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 #1

The manuscript does address relevant scientific questions within the scope of BG. It also presents partly novel data.

Are there substantial conclusions reached or not is questionable as the manuscript intends to show results from feeding experiments that where obviously to short or failed. *N. labradorica* is usually a deep infaunal (limited by the availability of oxygen) dwelling species that covers its test with a sedimentary cyst.

We thank the reviewer for her constructive comments.

We agree with the reviewer that the feeding experiment was too short and should have been longer. We agree that longer experiments would indeed be good to possibly stimulate feeding uptake by these specific Arctic foraminifera, especially because their movement to approach prey also seemed reduced. However, there is no basis for assertion, to the statement that *N. labradorica* is a deep infaunal (limited by the availability of oxygen). It is clearly infaunal, but was in our collection sampled in the top 1 cm. Our analysis of TEM pictures did not conclude that they are limited by oxygen (e.g. number of mitochondria in the cell), where they lived. If the reviewer has any (un)published data or suggestions for further literature source on that, we are willing to include it.

In these experiments the sedimentary cyst was removed, which should have led to significant stress for the animals as thereby also a significant amount of ectoplasm is removed.

It has not been established that cyst removal is detrimental to foraminifers. Further, it is likely that the foraminifers withdrew their pseudopodia upon the physical disturbance of the coring effort. Finally, we noted cytoplasm in every final (last formed) chamber of the TEM observations in this study, so any loss was likely insignificant.

We have added a paper, which clearly showed the occurrence and foramation of cysts (or sedimentary envelops) around foraminifera is a common feature and not only related to stressful conditions. It has been added in the methods section.

Line 158-161 Living specimens had a partly inorganic covering surrounding the test, which was gently removed using fine artist brushes. Those so-called cysts are nothing unusual with many groups of foraminifera (Heinz et al., 2005).

Being placed in petri dishes with artificial seawater and after what is called a starvation period of 2-4 hours **(why this range?)**, that from my experience is too short for foraminifera specimen to recover, either cultured *M. sedimenti* was added or not. The maximum experimental incubation time was 20 h. Of the 17 specimens selected only 2 specimens showed very a total of 3 supposed methanotrophic bacteria close to the reticulopodial network that rests in the final chamber of rotaliids. Obviously, none of the identified bacterial remains could be definitively related to the provided bacterium. Furthermore, obviously all specimens showed clay particles in the final chamber, that was not provided during the experiment. The fact that clay was observed in the last chamber of all specimens and only 2 specimens showed 3 bacteria that eventually from which two were not even provided during the experiment, indicate that clay and bacteria were eventually in place before the start of the experiment. Although I consider it logical that *N. labradorica* may facultatively nourish on bacteria, the experiments are no proof. Rotaliids extend and retreat there pseudopodial network into the final chamber and whatever attaches to these filaments will be found there. If we look at the colorful Fig. 2a, it becomes obvious that we would expect any nutritious material predominantly in the older test parts (see also Wollenburg et al, 2018 Fig. 8b). The final chamber is a place of activity like a garage for pseudopodia or for short storage, but if you don't find traces of the provided bacteria in older chambers, their presence in the last chamber could just be accidental. Support for this suggestion comes from the very few observations of bacteria and the absence of *M. sedimenti*.

We agree with the reviewer that the nutritious material is to be expected in the older test parts, which are filled with cytoplasm (see Fig. 2a) and not in the final chamber. To our understanding the final chamber is also the place of activity of the pseudopods, as we saw remains of those in the TEM pictures. In the final chambers not all organelles are present, as they are located mainly in older parts of the cell. However, we think that during the time a "food vacuole" takes to get to the third chamber, is too long for any bacteria or food sources to be identifiable. To our knowledge no study has timed the transport of "food" from the outside of the foram to the older chambers.

So, I consider the experiment as too short or rather failed and **would focus the manuscript on the great TEM figures and place some speculative assumptions on experimental pictures** (by the way I like the pictures), or to **redo the experiments for a significantly longer period of time** and with labelled bacteria. This would also proof that the specimens even survived the treatment, a 24-h experiment on specimens that immediately died during the treatment would show no different TEM pictures, and as no pseudopodial activity was reported, the survival rate is unknown.The scientific methods are sufficiently described and can be reproduced by scientists.

We agree with the reviewer that the starvation phase of 2-4 h is too short. Hence, we rename "starvation" to "pre-adaptation phase" to experimental conditions. We could not possibly extend this duration, as specimens were not transferred to a laboratory on land. *Nonionellina labrarodica* is sensitive to both light and temperature (Jauffrais et al. 2019), so we choose to conduct the experiment onboard the vessel. We agree with the reviewer that the term starvation phase is rather misleading, as the results showed that foraminifera did not starve during this time, as degradation /food vacuoles were still full of sediment after the 20-h incubation phase. We have done following adjustments in the text:

(Old manuscript text lines 193-195) The 20-h feeding experiment with *M. sedimenti* started after a short starvation phase where organisms resided in petri dishes with ASW for 2-4 h and were not fed or manipulated during this time.

New manuscript text lines 200-208: The experiment with *M. sedimenti* was conducted for the total duration of 20-h to resemble previous experiments on *N. labradorica* on transmission electron microscopy and nanometre-scale secondary ion mass spectrometry (TEM-NanoSIMS) isotopic imaging (Jauffrais et al. 2019), and included two more time points at 4 and 8-h, where incubations were terminated.

A short pre-experimental phase (2-4 h) was included before the initial start of the feeding experiment, to allow specimens to recover. During the pre-experimental phase specimens were not fed and resided in the petri dishes to adjust to the experimental conditions.

I don't consider the experimental results sufficient to support the interpretations and conclusions, but **I would like to see a revised version focusing on the TEM pictures** especially from the TEM pictures with the **paired clay and bacteria vacuoles.**

We agree with the reviewer, that more detailed illustration of the paired clay and bacteria vacuoles would be helpful. We have therefore included a new Figure 6 in the manuscript, which shows six panels of clay and bacteria instead of only three in the first manuscript draft.

The Figure caption reads as follows:

Fig. 6. TEM micrographs of *N. labradorica* showing degradation vacuoles containing miscellaneous items, including bacteria (b), inorganics (clay platelets) and unidentifiable remains after 4h incubation (a,b; specimens E27, E28, respectively); after 8h incubation (c,d; specimen E14), after 20h incubation (e,f; specimens E36, E37, respectively). v=vacuole, dv=degradation vacuole, c=kleptoplast, p=peroxisome, m=mitochondrion, li=lipid, g= Golgi. Scales: (a, c-f) 1 µm, (b) 2 µm.

I don't understand why the authors elaborate on geochemistry methods, when neither d13C or other geochemistry aspects have been applied to the investigated specimens. **I would recommend to either get rid of this method chapter, except for the basic oceanographic feature or to put them in context to the foraminiferal data. A basic information on the sedimentary composition at the coring site is needed to put the clay particles in context.**

In the paragraph above we think the reviewer refers to the methodology chapter 2.2 (line 148 old manuscript 2.2 Geochemistry) which clearly deliberates the Geochemistry of the site. This is in our opinion important as the core PUC2 served as a reference core, which describes that our site was an "active methane seepage site". We have in the revision name the section: 2.2 Geochemistry of the study site. It is hence more specific and the reader understands that we are discussing the geochemistry of the study site and not of foraminifera tests. We do not have basic information of the sedimentary composition at the coring site, to put the clay particles in the context, but we agree with the reviewer that this would be very nice to do. Anything related to the clay particles has been commented on in the next comment.

The data on the geochemistry PUC2/F7.and another image of the sampling location for it has been placed into Supplementary Table 1.

Results: Line 259 old manuscript: At approximately 13 cm the sulfate measured in the pore water of the geochemistry core (PUC2) declined from ~2750 ppm at the sediment-water interface to ~706 ppm (see Fig. S1, Table S1).

Furthermore, is there a preferred grain size spectrum in the food vacuoles? If yes, why? What does this tell us.

We like to note that the grain size varies depending on the orientation of the grains embedded in the resin and can be easily biased depending on the direction of sectioning with the knife.

The description of experiments is sufficiently complete and precise to allow their reproduction by fellow scientists.

I think that the authors should leave their inner circle of referencing. Especially **regarding carbon isotopes there is a misinterpretation of published work that essentially relies on the assumption that foraminiferal shells reflect the bottom or pore water signature they are dwelling in not their nutrition. And regarding Wollenburg et al (2007) the statement they place is wrong.** This study confirmed that foraminiferal shells reflect the emanation of methane and its signature. The authors usually give proper credit to related work and clearly indicate their own new/original contribution.

The authors have extended the scope of referencing by including Wollenburg (2015). We think the reviewer was mentioning that paper, and changed the statement we place in relation to it.

Old manuscript lines 69-73: Experiments using a novel high-pressure incubator on Cibicides wuellerstorfi illustrated the difficulty to measure the relationship between methane exposure, δ13CDIC and δ13Ctest, as whole cores were incubated, the δ13CDIC of the seawater was impossible to keep constant and to compare δ13Ctest formed in the presence of methane to normal marine conditions (Wollenburg et al., 2015).

Line 62-65 Experiments using a novel high-pressure incubator illustrated the difficulty to measure the relationship between methane exposure and the foraminifera *Cibicides wuellerstorfi*. However, it was shown in this experiment using entire cores, that the methane source is reflected in δ^{13}Ctest of foraminiferal calcite (Wollenburg et al., 2015).

The **title** clearly reflects the contents of the paper, **but should be addressed to *N. labradorica* only**

The title has been addressed to *Nonionellina labrarodica* only, and has been changed to avoid overinterpretation of results regarding the possible association with methanotrophs (Suggestion of Reviewer 2) to the following.

Deposit-feeding of *Nonionellina labradorica* (foraminifera) from an Arctic methane seep site and possible association with a methanotroph revealed by transmission electron microscopy

The **abstract has to be shortened** to be concise.

Abstract was shortened by several characters /words to be more concise. We have reduced the abstract from 320 words to 220 words (-100 words, 32%) and 30% in characters incl. spaces (from 2294 to 1606).

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 δ^{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 ultrastructural 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.

The overall presentation is not well structured and should be **streamlined.**

The overall paper was re-read by all authors and streamlined. Several examples for streamlining are that the abstract was made more concise and the method section improved by the reviewer comments.

The language is fluent.

The whole text contains a lot of repetitions and should be rewritten in a way that one aspect is addressed predominantly at only one place in the introduction and discussion. **There are also a lot of typos and missing blancs.**

Typos and missing blancs and commas have been removed as good as possible, and the manuscript automatically spell-checked. The introduction has been made precise when talking about studies citing influence of methanotrophs on the delta 13C values.

Studies specifically looking at living (bengal rosa stained) foraminiferal tests support the hypothesis that the carbon isotopic composition is strongly influenced by the porewater DIC (McCorkle et al., 1990a). Interspecific $\delta^{13}C$ differences between species with similar depth indicate sometimes taxon-specific “vital” effects (McCorkle et al., 1990a). Those “vital” effects describe the biology of the different species, which could reflect different feeding patterns. It has been suggested that *Nonionella auris* is an indicator of methane and possibly ingested ^{13}C-depleted methane oxidising bacteria, which were the reason for the (Wefer et al., 1994).

We have streamlined the introduction, and removed a lot of redundant words.

The supplementary material could be improved by showing successful **TEM pictures of all investigated specimens.**

The TEM pictures are very large for the supplementary material. Hence we uploaded them to the PANGAEA platform. They will be available upon publication having their own DOI and citable on their own. They can also be shown to the reviewer, if requested before the date they are publicly available.

References

Jauffrais, T., LeKieffre, C., Schweizer, M., Geslin, E., Metzger, E., Bernhard, J.M., Jesus, B., Filipsson, H.L., Maire, O., Meibom, A., 2019. Kleptoplastidic benthic foraminifera from aphotic habitats: insights into assimilation of inorganic C, N and S studied with sub-cellular resolution. Environmental Microbiology 21, 125–141. https://doi.org/10.1111/1462-2920.14433

Heinz, P., Geslin, E., and Hemleben, C.: Laboratory observations of benthic foraminiferal cysts, Mar. Biol. Res., 1, 149-159, 2005.

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 #2

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 weak results.**

We agree with reviewer 2 that 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 have included the scientific name in the Title as this was requested by Reviewer 1

Deposit-feeding of *Nonionellina labradorica* (foraminifera) from an Arctic methane seep site and possible association with a methanotroph revealed by transmission electron microscopy

The original scientific question of the manuscript fits into the field of biogeosciences, **but the results presented do not fulfill 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.**

We agree with the reviewer that the manuscript fits into biogeosciences topic, as it is a relevant topic to better understand feeding behavior of benthic foraminifera, which could ultimately have an influence on the isotopic signature of delta^{13}C of their tests.

The concept and the data presented in this manuscript are of novel nature. Very few published papers have shown data from living foraminifera from active methane emission site from the Arctic. On this species no other feeding experiment has been performed to date, and all images presented are original. Furthermore, no ultrastructure of deep water N. labradorica has been presented to our knowledge. All other published works used specimens collected shallower from Gullmar Fjord (Sweden) sediment samples were collected from a depth of ~ 70 m using a box-corer (Jauffrais et al. 2019).

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.**

We note that the methanotrophs were once isolated from a whale fall isolated from sediment, and not methanotrophs from the water column, as Reviewer 2 understood.

We like to keep the geochemical analysis in the manuscript, as they are important to show that the site, where foraminifera were collected, was classified as an active methane emission site. The molecular genetics is used because it is the modern means to identify a species. For foraminifera, this is typically done with both morphology and sequencing. However, the reviewer is correct that we do not show the data enough, and have changed this. We deem it is sufficient to include the data in the Supplementary material.

1. Geochemical analysis: A Table has been added to the supplementary material showing the sulfate, DIC of the pore water of the geochemistry core. This was collected 5 m away from the actual sampling site, which contained the blade corer and the living foraminifera. The blade corer where the living foraminifera originated from was not possible to be analyzed for geochemistry.
2. Molecular data: The molecular sequences uniquely identify the species as *Nonionellina labradorica.* However, as there are cryptic species, and in order to relate the work to future work, the sequence has been published and was referenced already in the old version of the manuscript. Hence, we do not see the need to change or extend discussion of it.

Reviewer 2 asked why we collected foraminifera from the 1-2 cm in the blade corer. That is because this species was abundant at this depth in the blade corer sample. Determining the sulfate decline and DIC of the pore water let us conclude that this is an active site.

- 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 reviewer is right about this, our initial plan was also to label the bacteria with a biomarker /label. However, there was a last-minute issue so this was not possible.". The first approach in this study was to investigate whether this foraminifera would feed on bacteria, and this we can clearly answer with the presented data. It turned out that next to the association with bacteria, the foraminifera are strong deposit feeders. This would have never been noted without close examination viaTEM, as presented.

- The **incubation time seems to have been planned too short**, especially at these cold temperatures, because no uptake was observed.

We agree with the reviewer that the incubation time was rather short and longer experiments may have shown uptake. However, based on previous experiments that incubate this species with 13C and 15N isotopes at low temperature (Jauffrais et al. 2019) recording metabolism activities after 20 hours, it was logical that we used the same incubation duration. We agree that longer experiments would indeed be good to possibly stimulate feeding uptake by these specific Arctic foraminifera.

(Line 193-195) The 20-h feeding experiment with *M. sedimenti* started after a short starvation phase where organisms resided in petri dishes with ASW for 2-4 h and were not fed or manipulated during this time. The experiment with *M. sedimenti* was conducted for the total duration of 20-h to resemble previous experiments on *N. labradorica* using transmission electron microscopy and nanometre-scale secondary ion mass spectrometry (TEM-NanoSIMS) isotopic imaging (Jauffrais et al. 2019), and included two more time points at 4 and 8-h, where incubations were terminated. A short pre-experimental phase (2-4-h) was included before the initial start of the feeding, to allow specimens to recover from the experimental setup. During the pre-experimental phase specimens were not fed and resided in the petri dishes to adjust to the experimental environment.

- 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 starvation period was chosen to be within 2-4 hours because we wanted to not expose the specimens longer than needed before the actual start of the experiment, and risk their death. A pre-adaptation phase of several days was not feasible in this study. However, as those Arctic specimens are sensitive to both light and temperature we choose to conduct the experiment onboard the vessel. Because of logistics and the entire duration of the cruise, the starvation phase was chosen to be rather short (2-4h). As the results showed that foraminifera did not starve during this time, as they were still having full food vacuoles, we re-name this phase as an pre-experimental phase, where organisms are supposed to adjust to experimental conditions.

(Line 193-195) The 20-h feeding experiment with *M. sedimenti* started after a short starvation phase where organisms resided in petri dishes with ASW for 2-4 h and were not fed or manipulated during this time. The experiment with *M. sedimenti* was conducted for the total duration of 20-h to resemble previous experiments on *N. labradorica* on transmission electron microscopy and nanometre-scale secondary ion mass spectrometry (TEM-NanoSIMS) isotopic imaging (Jauffrais et al. 2018), and included two more time points at 4 and 8-h, where incubations were terminated. A short pre-experimental phase (2-4-h) was included before the initial start of the feeding, to allow

specimens to recover from the experimental setup. During the pre-experimental phase specimens were not fed and resided in the petri dishes to adjust to the experimental environment.

- The authors mention that for each experimental time point, 4 out of 5 foraminifera were examined. **Was the selection for these 4 purely random?**

Yes, the selection of samples was purely random. We selected the specimens presenting the better orientation in the resin block to facilitate TEM observation of the chambers of interest. . Hence, the four samples were chosen which we were able to cut through the aperture region. I have added this in the methods section.

Old Manuskript: Samples preserved in fixative solution were transported to the University of Angers, where they were prepared for ultrastructural analysis using established protocols (Lekieffre et al., 2018). Embedded foraminiferal cells were sectioned using an ultramicrotome (Leica® Ultracut S) equipped with a diamond knife (Diatome®, ultra 45°). Grids were stained using UranyLess® EM Stain (EMS, USA).

New Manuscript Lines 189-194: Samples of *N. labrarodica* preserved in fixative solution were transported to the University of Angers, where they were prepared for ultrastructural analysis using established protocols (Lekieffre et al., 2018). Four embedded foraminiferal cells per treatment were sectioned using an ultramicrotome (Leica® Ultracut S) equipped with a diamond knife (Diatome®, ultra 45°). Grids were stained using UranyLess® EM Stain (EMS, USA).

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 reviewer is right that the degradation vacuoles containing clay/ inorganics originate from particle engulfment that took place before the incubation, when specimens were still in sediments. We analyzed 17 samples, 12 fixed after incubation and feeding on bacteria (for 4-20-h), and 4 fixed directly after field collection , and 1 as a non-fed control (fixed after an incubation of 20-hours). In all food vacuoles the sediment was present irrespective of treatment. This is shown in Table 1.

As reviewer 2 pointed out, the degradation vacuoles containing clay particles were formed before the experiment and thus cannot be linked with the ingestion of methanotrophs. However they still demonstrate the deposit-feeding trait of *N. labradorica* species. Modifications have been made in the text to clarify this point.

It has been adjusted in the Title: Deposit-feeding activity of *Nonionellina labradorica* (foraminifera) at an Arctic methane seep site and putative association with a marine methanotroph

- 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.**

We do not agree with reviewer 2 that foraminifera were not cleaned properly before they were placed in the petri dishes. We have cleaned them with several strokes of fine artist brushes and took great care to not damage their delicate test. Also please note that the association was only observed in the 20-h incubation. Hence, we strongly believe that the foraminifera really approached the bacteria. Furthermore, one of the putative methanotrophs had characteristic ICM (Intracytoplasmic membranes), which resemble the methanotroph *M. sedimenti* in culture. Cleaning the foraminifera with another means, such as ethanol bath would have been too risky to not damage the microbiome around the foraminifera.

However, we agree to the reviewer that this is a valid point, and it needs discussion. The sentence in the discussion has been changed accordingly.

Line 323, 358, 359 The association has been changed to "the possible association"

The paragraph of the discussion "4.2 Possible association with putative methanotrophs" has been shortened from 295 words by 47% to be 168 words, as the discussion on the SMTZ and AOM (Anaerobic oxidation of methane) was removed based on reviewer 1 comments for adjusting introduction and discussion.

4.2. Possible association with putative methanotrophs

The possible association with the three putative methanotrophs was identified on two specimens based on comparing internal characteristics of methanotrophs (Tavormina et al., 2015) to our images. The documentation of this possible association with putative methanotrophs likely is due to the methanotrophs given as food in the experiment. However, there is a small possibility that the associated methanotrophs stuck on the outside of the test and could be remaining from the field, as foraminifera were carefully cleaned but not washed in ethanol before the start of the experiment to preserve its microbiome. The results of our observation match to the result of observations on *Melonis barleeanus* (Bernhard and Panieri, 2018), where a putative association of foraminifera and methanotrophs has been described recently originating from the field. However, the deposit-feeding behavior ingesting large amounts of sediment, which was described in this study for this species for the first time, shows that methanotrophs can be ingested via untargeted grazing in seeps, as N. labradorica appears to be a non-selective feeder.

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.

We agree with ther reviewer that the introduction should not focus on $\delta^{13}C$ signals alone as those were not measured. However we think it is important to discuss them, as they would be ultimately influenced by different food sources. The text of the introduction has been shortened by 1.5 paragraphs (old manuscript Line 74-87). The sentence about the MDAC, ws kept.

Several studies found that the lightest isotopic $\delta^{13}C$ values were measured in tests coated by methane-derived authigenic carbonate (MDAC) overgrowth, which happens after the death of the protist (Torres et al., 2010; Panieri et al., 2014; Consolaro et al., 2015; Panieri et al., 2017; Schneider et al., 2017).

The introduction has been made precise when talking about studies citing influence of methanotrophs on the delta 13C values.

Studies specifically looking at living (bengal rosa stained) foraminiferal tests support the hypothesis that the carbon isotopic composition is strongly influenced by the porewater DIC (McCorkle et al., 1990a). Interspecific $\delta^{13}C$ differences between species with similar depth indicate sometimes taxon-specific "vital" effects (McCorkle et al., 1990a). Those "vital" effects describe the biology of the different species, which could reflect different feeding patterns. It has been suggested that *Nonionella auris* is an indicator of methane and possibly ingested ^{13}C-depleted methane oxidising bacteria, which were the reason for the (Wefer et al., 1994).

Concerning the supplementary material, I **miss the data of the geochemical measurements.**

We agree with Reviewer 2, that due to revisions of the manuscript the Table showing the geochemistry measurements had been removed. It has been added in the Supplementary Table 1, showing the geochemistry of the PUC2/F7 and an image of this associated core.

Results: Line 259 old manuscript: At approximately 13 cm the sulfate measured in the pore water of the geochemistry core (PUC2) declined from ~2750 ppm at the sediment-water interface to ~706 ppm (see Fig. S1 and Table S1).

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.

We believe that the readership of biogeoscience will find that manuscript interesting as it is an interdisciplinary work between microbiology and foraminiferal research. The study looks to increase our understanding of feeding habits of foraminifera on the sea floor around active methane seepage in a geochemically active site, which is interesting for readers of BG. We hope that Reviewer 2 points were sufficiently addressed, and that if open questions remain they can be resolved to publish this manuscript in the BG journal.

References

Jauffrais, T., LeKieffre, C., Schweizer, M., Geslin, E., Metzger, E., Bernhard, J.M., Jesus, B., Filipsson, H.L., Maire, O., Meibom, A., 2019. Kleptoplastidic benthic foraminifera from aphotic

habitats: insights into assimilation of inorganic C, N and S studied with sub-cellular resolution. Environmental Microbiology 21, 125–141. https://doi.org/10.1111/1462-2920.14433

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 sedimenti* 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.