

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 were obviously too 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 formation of cysts (or sedimentary envelopes) 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 their 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 prove 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 labradorica* 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 d^{13}C 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, $\delta^{13}\text{CDIC}$ and $\delta^{13}\text{C}_{\text{test}}$, as whole cores were incubated, the $\delta^{13}\text{CDIC}$ of the seawater was impossible to keep constant and to compare $\delta^{13}\text{C}_{\text{test}}$ 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 $\delta^{13}\text{C}_{\text{test}}$ 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 labradorica* 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 $\delta^{13}\text{C}$ 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.

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 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. Kleptoplastic 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.