

## 2nd round of Revisions for BG-2021-284

We thank the reviewer for their repeated 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)

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### Report of Reviewer #1

**The revised manuscript has improved considerably. Most reviewers' comments and suggestions were considered. It is now more focused on the deposit-feeding character of *Nonionellina labradorica* and less emphasized on the feeding experiment. More detailed TEM pictures were added. The text is more streamlined.**

### Some additional minor comments:

**You sometime use the term ICM for intracytoplasmic membranes, sometimes for intracellular stacked membranes, and sometimes you call intracytoplasmic stacked membranes ISM, but you mean always the same structure? It is quite confusing.**

Answer: In the publication (Tavormina et al., 2015) it is referenced as typical type I stacked intracytoplasmic membranes (ICM). They are of course also intracellular but this was rather a mistake from our side, and I have corrected it in the final manuscript. Also I have streamlined the text as some sections from line 241-247 where repetitive.

New Figure Caption:

Figure 1 Transmission electron micrographs of *N. labradorica* from 20 h treatment (sample E39) (a) Stitched cross section of TEM images showing location of methanotroph at the aperture region (black rectangle) (b) Location of two putative methanotrophs next to sediment particles and putative reticulopodial remains. (c) Close up of two putative methanotrophs revealing detailed feature for identification, such as typical type I stacked intracytoplasmic membranes (ICM), and other less-informative characteristics, such as storage granules (SG), and gram-negative cell wall (GNCW), scale bars: a: 100  $\mu$ m, b: 1  $\mu$ m, c: 200 nm.

Old text Line 241-247:

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 appear to have a gram-negative cell wall, coccoid to slightly elongated shape

and characteristic intracytoplasmic membrane (ICM) (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) (Fig. 2).

**New text:**

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 are coccoid to slightly elongated shape and is characterized by typical type I stacked intracytoplasmic membranes (ICM) (Fig. 2c). It has storage granules (SG) and a gram-negative cell wall (GNCW), which are not uniquely charactersitic of methanotrophs (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).

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**Line 125-126: twice?:.....reviewed in ( reviewed in ...)**

It has been corrected and reads now.....herbivorous, carnivorous, suspension feeders and most commonly deposit feeders (reviewed in Lipps, 1983).

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**Line 143: What do you mean with specific ornamentation at the aperture, some kind of pustules? Can you give more details, maybe also referring to your supplement Figure S4.**

Answer: Indeed we mean the pustules and teeth observed in Bernhard and Bowser 1999. Our SEM images are not detailed enough presented in Figure S4, as they are only for ID purposes, hence we cannot refer to them in this context.

**Old text:**

*Nonionellina labradorica*'s aperture shows a specific ornamentation, possibly a morphological adaptation to this "predatory" mode of life for obtaining the kleptoplasts (Bernhard and Bowser, 1999).

**New text:**

*Nonionellina labradorica*'s aperture shows a specific ornamentation, possibly a morphological adaptation to its "predatory" mode of life. It hosts "teeth" extending in the aperture and pointed tubercles, which could play a role by liberating the chloroplasts from the algal prey (Bernhard and Bowser, 1999).

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**Line 145:... reviewed in Charrieau...**

The reviewr is right, there it was a typing mistake and corrected.

**Line 230: Why you cancelled this reference (Jauffrais et al., 2019) here?**

The reviewer is right, that was due to an oversight on my side. I included Jauffrais et al. 201b again.

It has to do that there is two papers published by Jauffrais in 2019 and both have now been included in the manuscript.

Here we refer to following study:

(Jauffrais et al., 2019b) Jauffrais, T., LeKieffre, C., Schweizer, M., Geslin, E., Metzger, E., Bernhard, J. M., Jesus, B., Filipsson, H. L., Maire, O., and Meibom, A.: 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>, 2019.

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**Fig. 6 b: Indicate where the enlarged picture is coming from.**

We agree with the reviewer that it is maybe not entirely clear if the Figure 6 b) is not viewed in full size. Hence, we decided to indicate in the Figure caption that enlarged picture is a zoom window to see unidentifiable remains in better detail. We hope that this is making it clear without changing the image itself.

Figure 2 TEM micrographs of *N. labradorica* showing degradation vacuoles containing miscellaneous items, including bacteria (b), inorganics (clay platelets) and unidentifiable remains after 4h incubation, **which are shown enlarged in the left side of the image in a zoom window** (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  $\mu\text{m}$ , (b) 2  $\mu\text{m}$ .

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**Line 400: Add a sentence at the end of this chapter 4.1. that with this geochemical data, you show that the site, where living foraminifera were collected, was classified as an active methane emission site.**

**We agree with the reviewer that the point of including this paragraph at the beginning of the dicussion is to show that the sample was from an active methane emission site. Hence, we included a sentences with this wording at the end of chapter 4.1.**

New text:

**The geochemical data at PUC2 let us conclude that the site, where living foraminifera were collected, can be classified as an active methane emission site.**

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**Line 413-414: Can you maybe give some more information here about that putative association of *Melonis barleeanus* with methanoptophs?**

Yes indeed the reviewer is right, that the old text did not specifically give enough information besides referring to the paper (Bernhard and Panieri, 2018). This has been corrected, and more detailed explained in the new text by adding minimally more words (characters).

**Old text:**

Our results are similar to observations on field-collected *Melonis barleeanus* (Bernhard and Panieri, 2018), where a putative association with methanotrophs was described.

**New text:**

Another benthic foraminifer, *Melonis barleeanus*, has been noted to have clumps of putative methanotrophs at the apertural opening of field-collected specimens

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**Supplement Table S1: ...The entire profile of this core indicates that...**

It has been corrected and the typing mistake in the word indicate has been removed .