

Dear reviewers, Dear Lisa Levin (Editor),

thank you very much for the feedback to this review paper. I am really glad that three experienced specialists from this field provided such detailed constructive revisions to the manuscript. Of course, a review article will strongly benefit from discussing different opinions and different points of view of several experts. I revised my manuscript thoroughly regarding the feedback to all three reviewers. Below you can find a detailed point by point response to the review by Anonymous Reviewer#2.

**Reviewer:** The manuscript by Glock is a good initial attempt to review current knowledge about benthic foraminifera inhabiting anoxic AND oxygen-depleted (hypoxic, dysoxic) habitats. In fact, roughly half of the situations described regard foraminifera living in low – perhaps even moderately high— oxygen conditions. Further, manuscript portions discuss gromiids / *Gromia*, which are not foraminifera. With these two things in mind, the present title must be changed in terms of “anaerobic” and “foraminifera”. In particular, the title must include low oxygen (or synonym) and “gromiids” or the contribution must be stricken of all discourse about low-oxygen habitats and the gromiids. Further, the use of plural (“Reviews and syntheses”) is grammatically incorrect. In my opinion, the title should also include “benthic” as there are only a few sentences about planktic foraminifers. The abstract too requires edits after considering the detailed comments that follow.

**Reply:** Regarding the plural in “Reviews and syntheses”: A similar recommendation has been given by another reviewer (Frans Jorissen). I agree with these statements and did not have “Reviews and syntheses” in my original title. After my initial submission but before the upload of the preprint I had the request by the editorial office to change my title accordingly. “Reviews and syntheses” is mandatory in the title of review papers that are submitted to this journal.

For the rest: I added “Benthic foraminifera” to the title and changed “anaerobic” to “oxygen depleted” to be less restricting. I am not opposed to add “gromiids” as well. I also clarified now specifically in the text that gromiids aren’t foraminifera, when gromiids are first mentioned in the text:

“Other studies showed that bacterial endobionts likely perform denitrification in some allogromiid foraminifera and gromiid species (Bernhard et al., 2012a; Høglund et al., 2017). Gromiida are a separate group of protists within the Rhizaria and closely related to foraminifera.”

In addition, I separated foraminifera and gromiids in table 2.

I would change the title of the paper as follows, which also addresses the points of revision by the other two reviewers:

“Reviews and syntheses: Benthic foraminifera and gromiids from oxygen depleted environments - Survival strategies, biogeochemistry and trophic interactions”

**Reviewer:** It is not clear why certain topics received much attention while other “survival strategies” for inhabiting oxygen-depleted habitats were effectively ignored (symbionts, for example).

**Reply:** I discussed symbiosis in several parts of the “survival strategies chapter” (examples see below). It was certainly not my intention to ignore this point. Due to the statement by the reviewer, I assume that this discussion was not comprehensive and to address this issue, I added the following parts to the revised manuscript:

“There is strong evidence for symbiosis between foraminifera and prokaryotes in many hosts from O<sub>2</sub> depleted environments, which most likely are an adaptation to survive within the steep geochemical gradients close to the oxic/anoxic boundary (Bernhard et al., 2000; Bernhard, 2003; Bernhard et al.,

2006; Nomaki et al., 2014; Bernhard et al., 2018). Most of the observed prokaryotic associates are endobionts within the foraminiferal cytoplasm but some are ectobionts that often are observed close to the pores in the foraminiferal shell (Bernhard et al., 2001, 2010a, 2018). For about a decade after the first discovery of foraminiferal denitrification it remained unclear if foraminifera indeed denitrify themselves, or if the bacterial symbionts are responsible for the denitrification. Evidence came up for both hypotheses.”

“Recent metagenomics and transcriptomics results of denitrifying foraminifera indicate that bacterial symbionts might perform the missing steps in the foraminiferal denitrification pathway or that they at least partly contribute to the amount of  $\text{NO}_3^-$  that is denitrified within foraminiferal cells (Woehle & Roy et al., 2022). It has already been hypothesized before that the ectobionts, found on *Bolivina pacifica* from the Santa Barbara Basin are either sulfate reducing or sulfur oxidizing bacteria (Bernhard et al., 2010a).”

“A continuum of intracellular bacteria including prey in food vacuoles, endobionts, parasites and necrophages has been documented before in benthic foraminifera from cold seeps (Bernhard et al., 2010b). It already has been hypothesized by the authors that bacteria switched their function from endobionts to predators, depending on the vitality of the host cell.”

In addition, two other studies that discuss alternative adaptations of foraminifera to  $\text{O}_2$  depletion (Bernhard & Bowser, 2008 & Powers et al., 2022) are now discussed in a bit more detail in the manuscript:

“Most foraminifera species from  $\text{O}_2$  depleted habitats possess numerous peroxisomes that are usually associated with mitochondria and the endoplasmic reticulum (Bernhard and Bowser, 2008). Bernhard and Bowser (2008) hypothesized that these peroxisome proliferations might be used to either metabolize  $\text{H}_2\text{O}_2$  and other highly reactive oxygen species that are produced within the chemocline close to the oxic/anoxic boundary or to reduce the oxidative stress by these compounds. Indeed, they showed in an experiment that ATP concentrations in foraminifera increased proportional to ambient  $\text{H}_2\text{O}_2$  concentrations. A recent study on transcriptome and metatranscriptome of *N. stella* and *B. argentea* from the Santa Barbara Basin revealed that these species utilize an adaptable mitochondrial and peroxisomal metabolism, depending on the chemical treatment in the experiment (Powers et al., 2022). The high plasticity of their peroxisomal and mitochondrial metabolism might be substantial for survival at the highly variable conditions at the chemocline in the sediments. The results by Powers et al. (2022) indicate that at least some processes that are involved in foraminiferal denitrification are associated with mitochondria. Interestingly, the expression of denitrification related genes in both species was upregulated after incubation with elevated  $\text{H}_2\text{O}_2$  but without  $\text{NO}_3^-$  and downregulated, if they were incubated without  $\text{H}_2\text{O}_2$  but with  $\text{NO}_3^-$ , compared to a control treatment with both  $\text{H}_2\text{O}_2$  and  $\text{NO}_3^-$ . In the same way several peroxisomal processes were upregulated in the  $\text{H}_2\text{O}_2$  only treatment. In addition, despite that both species are able to denitrify, Powers et al. (2022) found distinct metabolic adaptations to anoxia in both species. For example, a quinol:fumarate oxidoreductase, which is considered as an adaptive mechanism for anaerobic respiration in eukaryotic organisms, was present in *N. stella* but not in *B. argentea*. Vice versa, *B. argentea* has the capacity to digest food vacuole contents under  $\text{O}_2$  depletion, while *N. stella* was lacking food vacuoles (Powers et al., 2022).”

As I mentioned above, I did not ignore symbiosis in the original manuscript and it was not my intention to ignore any of the present studies. Here are examples, where I already discussed symbiosis in the original manuscript:

Line 90-95: “For about a decade after the first discovery of foraminiferal denitrification it remained unclear if foraminifera indeed denitrify themselves, or if they host **bacterial symbionts** that are responsible for the denitrification Bernhard et al. (2012a) showed that *Bolvina argentea* was able to denitrify, even after a very harsh treatment with antibiotics, which indicates that this species can denitrify even, when potential **bacterial symbionts** are killed. Other studies showed that **bacterial endobionts** likely perform denitrification in some allogromiid species and gromiids (Bernhard et al., 2012a; Høglund et al., 2017).”

Line 100-104: “Nevertheless, the homologues of the enzymes that catalyze the first and the last step of foraminiferal denitrification (Reduction of  $\text{NO}_3^-$  to nitrite ( $\text{NO}_2^-$ ) and reduction of nitrous oxide ( $\text{N}_2\text{O}$ ) to  $\text{N}_2$  gas; fig. 3) have not been identified, yet. This indicates either that foraminifera use other enzymes to catalyze these steps, that they rely on **bacterial symbionts** for these steps or that they use an alternative denitrification pathway in general.”

Line 110-112: “Recent metagenomics and transcriptomics results of denitrifying foraminifera indicate that **bacterial symbionts** might perform the missing steps in the foraminiferal denitrification pathway or that they at least partly contribute to the amount of  $\text{NO}_3^-$  that is denitrified within foraminiferal cells (Woehle et al., 2022).”

**Reviewer:** In reality, there are few studies that have shown bonafide “complete denitrification” as Risgaard-Petersen et al. did in their 2006 paper. To date, the ‘omics have shown the process lacks the final step, so those studies technically have not shown “complete denitrification”. This point should be made clear and elaborated upon as necessary throughout the contribution.

**Reply:** This is a valid point. The incomplete foraminiferal denitrification pathway is discussed later in the paper but it might be confusing, if this is not clearly stated already at the beginning. I adapted the part where I introduce Risgaard-Petersen’s results as follows:

“The discovery by Risgaard-Petersen et al. (2006) was also the first evidence for complete denitrification in eukaryotic cells in general and it also showed that they likely take up  $\text{NO}_3^-$  from the surrounding pore water and store it within intracellular seawater vacuoles. Nevertheless, no later study could actually proof a bonafide “complete” denitrification pathway in foraminifera and the eukaryotic foraminiferal denitrification pathway is today considered to be incomplete (Woehle et al., 2018; Orsi et al, 2020; Gomaa et al., 2021; see discussion below). Other eukaryotes that are known to perform incomplete denitrification are the primitive eukaryote *Loxodes* (Finlay et al., 1983) and two species of fungi (Usuda et al., 1995).”

**Reviewer:** The contribution should include a short synopsis where definitions of the terms used in the manuscript are defined. For instance, the term “anaerobic” typically refers to a physiological or metabolic process, not a habitat—meaning, “anaerobic environments” is unconventional phraseology. As the author likely knows, there are a plethora of terms designating environments with low concentrations of oxygen; these must be defined as the author interprets. For example, when one “low oxygen” concentration was presented, it was really quite high (60  $\mu\text{M}$ ), so it really is necessary that details be included.

**Reply:** The use of “anaerobic” might indeed be misleading as there are different definitions for this term and “anaerobic environments” are often better referred as “hypoxia”. Nevertheless, also the “definitions” of “hypoxic” or “suboxic” conditions often show a broad  $\text{O}_2$  concentration range that

differs between various literature sources. Therefore I will avoid these terms throughout the text of the revised manuscript to avoid further confusion or misunderstandings. Every time when the paper uses the term “anaerobic” it now refers to a metabolic process. “Anaerobic environments” are now termed either O<sub>2</sub> depleted environments, or in some cases, when O<sub>2</sub> is absent in these environments as “anoxic environments” or “anoxia”. “Anoxia” and “anoxic” are now defined in the introduction within the following sentence:

“The present paper will sometimes along the text refer to “anoxia” or “anoxic conditions”, which are now defined as the absence of oxygen.”

**Reviewer:** There are a number of overgeneralizations that require literature support or the assertions much be curtailed. For example, lines 13-14 state that benthic foraminifera as a group can benefit from ocean deoxygenation. This is vastly overstated. While some species may compete well in very low oxygen to anoxia, there is little, if any, evidence to suggest the entire group will benefit from deoxygenation. A second example involves the genus *Bolivina*, which is considered a model “deep infaunal” taxon in Fig. 4 yet there is no universally accepted agreement that all *Bolivina* are deep infaunal / anaerobes. Lines 149-150 notes four genera that are designated “deep infaunal facultative anaerobes” yet all species of each of these genera have not been assessed in this context so this assertion is premature.

**Reply:** The parts addressed by the reviewer have been adapted. The part in the abstract (originally line 13-14) has been rewritten to make clear that not all benthic foraminifera will benefit from ocean deoxygenation:

“Benthic foraminifera are a group of protists that include taxa with adaptations to partly extreme environmental conditions. Several species possess adaptations to O<sub>2</sub> depletion that are rare amongst eukaryotes and these species might benefit from ongoing ocean deoxygenation.”

The part where I discuss the deep infauna states that some *Bolivina* spp. are examples for deep infauna. This is not wrong, since *Bolivina seminuda* for example can certainly be considered deep infaunal. *Bolivina spissa* is certainly a shallow infaunal species due to the fact, that it selectively feeds on fresh phytodetritus. Figure 4 used schematic representations of *Bolivina* individuals as an example for deep infauna. This might indeed be misleading, because certainly not all *Bolivina* species are deep infaunal. In the revised manuscript, I removed the *Bolivina* drawings from the figure, although I have to say that the figure was visually more appealing before. In addition, I adapted the last line about the deep infauna. Now the text does not provide genera as examples for deep infauna but species names and refers that these “might be considered as facultative aerobes”:

“Taxa belonging to the deep infaunal group that might be considered as facultative aerobes that prefer NO<sub>3</sub><sup>-</sup> over O<sub>2</sub> include for example *Valvulineria inflata* and *bradyana*, *Bolivina seminuda*, *Globbulimina pyrula* and *Cancris carmenensis* (e.g. Jorissen et al., 1995; Mojtahid et al., 2010; Glock et al., 2019c).“

**Reviewer:** Other statements are incorrect. For example, lines 66-68 note that foraminifera are the only eukaryote to perform denitrification. This is not true—a number of fungi also perform denitrification, and this was first described in the 1990s (Shoun et al. 1992 FEMS Microbiology Letters).

**Reply:** This is likely a misunderstanding. Of course, some fungi and for example also the primitive eukaryote *Loxodes* can perform incomplete denitrification. The part in 66-68 stated that “the discovery by Risgaard-Petersen et al. (2006) was also the first evidence for **complete** denitrification in eukaryotic cells in general...”. And up to date it is the only evidence for complete denitrification in

eukaryotic cells. Nevertheless, the reviewer is right that this study is also the only study that showed bonafide complete denitrification in foraminifera, yet. So this part has been adapted, according to my response of the earlier comment above and I also refer to the other eukaryotes that perform (incomplete) denitrification.

**Reviewer:** Also, the passage spanning lines 218 to 219 is simply wrong: Grzymski et al. (2003) – not Pillet et al., 2011— was the first to sequence foraminiferal kleptoplasts, documenting via molecular methods they were from diatoms. Further, the studies documenting kleptoplast morphology via TEM should not be discounted or belittled as diatom chloroplasts have distinctive morphologies. Molecular methods are not required to establish all facts.

**Reply:** My apologies for this unfortunate wording. It was neither my intention to discount or belittle morphological approaches, nor to discredit the author who sequenced the foraminiferal kleptoplasts before. The two sentences have been changed accordingly:

“The kleptoplasts in foraminifera originate from diatoms, which has been confirmed on the basis of the chloroplast shape in TEM-observations and by sequencing the chloroplasts with molecular biological methods (Lopez, 1979; Lee et al., 1988; Cedhagen, 1991; Lee and Anderson, 1991; Bernhard and Bowser, 1999; Grzymski et al., 2002; Goldstein et al., 2004).”

**Reviewer:** The fact that there is strong natural variability in nitrate storage causes the estimations of rates and contributions (lines 364) to be merely statistical manipulations that may mean little. This should be elaborated upon.

**Reply:** I disagree with the reviewer in this point. Yes, it is true that the nitrate storage and the metabolic rates of foraminifera show a wide range. This range is covered within the errors of the functions that are used to upscale to total budgets. These can be calculated, using classic statistical methods, such as propagation of uncertainty. I even stated this in the original text:

“Due to the high uncertainties related to the natural variability in metabolic rates and nutrient storage, a thorough error estimation is recommended (see Appendix B in Glock et al. 2020).”

Of course, we need more data to provide better estimates. I state this in several parts of the manuscript. For example:

“Given this variation in  $\text{NO}_3^-$  storage capability, the reliability of estimates for the foraminiferal contribution to  $\text{NO}_3^-$  budgets depends crucially on the availability of data. The more data there is, the better we are able to calculate foraminiferal  $\text{NO}_3^-$  budgets including the contribution of species with unknown denitrification rates or intracellular  $\text{NO}_3^-$ . ”

If the reviewer states that these are “merely statistical manipulations”, we need to bury a whole scientific discipline whose main tool is earth system modeling. For example, all “bottom-up” models that scale up different sources and sinks for different greenhouse gases, would be redundant! Models, that use knowledge about plankton ecology and chlorophyll distributions in the ocean to estimate global marine primary productivity: Redundant, since all kinds of phytoplankton species have different metabolic rates. Obviously, these models are often based on better studied groups of organisms. That’s why I cannot state enough that we need more “real” data to lower the uncertainty for estimating to total budgets. There are already biogeochemical models that successfully include active biological nitrate transport by foraminifera and sulfur bacteria and denitrification by foraminifera (e.g. Dale et al., 2016) and these will likely improve in the future.

**Reviewer:** Referring to lines 334-336, the author needs clarify why it is sensible to: (1) estimate the denitrification rate for species that are NOT documented to denitrify and (2) why it is sensible to think that the volume of a foraminiferal test reflects denitrification rate (think of the LBFs [Large Benthic Foraminifera] like *Amphistegina*—by this argument, it would have an extremely high denitrification rate, yet these species live on coral reefs which are not known for anoxic bottom waters).

**Reply:** It is obviously not my intention that researchers start to calculate a hypothetical nitrate storage for species that obviously do not denitrify, such as *Amphistegina* species that live on coral reefs. To clarify, why it is important to estimate these budgets also for the unknown species and that denitrification rates and nitrate storage should not be estimated for candidates that are unlikely to denitrify, I added the following part to the text:

“Given this variation in  $\text{NO}_3^-$  storage capability, the reliability of estimates for the foraminiferal contribution to  $\text{NO}_3^-$  budgets depends crucially on the availability of data. The more data there is, the better we are able to calculate foraminiferal  $\text{NO}_3^-$  budgets. Nevertheless, there are thousands of benthic foraminiferal species and a considerable amount of these species inhabit  $\text{O}_2$  depleted environments and potentially store  $\text{NO}_3^-$  and denitrify. It will be unrealistic to measure the intracellular nutrient content and metabolic rates for all foraminifera. Thus, functions to estimate the contribution of species with unknown denitrification rates or intracellular  $\text{NO}_3^-$  will provide more data for better estimates of total foraminiferal budgets within the nitrogen cycle. Of course, it is not possible to strictly define, which foraminiferal species are able to denitrify or to store  $\text{NO}_3^-$  without real measurements. If foraminiferal species inhabit  $\text{O}_2$  depleted environments and belongs to a genus of the species, listed in tab.1 or tab.2, as a rule of thumb, they are good candidates for potential denitrifiers. In addition, if a species is known to inhabit well oxygenated environments and/or belongs to a genus of the species shown in tab.3 it should be avoided to use equations presented below to estimate  $\text{NO}_3^-$  storage or denitrification rates.”

**Reviewer:** The section on kleptoplasty is mostly about species living in shallow, aerated environments— if there is proof of these species inhabiting anoxic conditions, that should be presented in this Review. It should be noted that the experimental conditions used by LeKieffre et al. (2018) were aerated (lines 223-225) so discussing this paper in the context of uptake during anoxia is misleading. Further, Jauffrais et al. (2019) did not incubate in anoxia either (Lines 245-246).

**Reply:** The reviewer is right, that a part of this section concerns kleptoplastic foraminifera from aerated environments and that some of the discussed experiments were done under aerated conditions. This is necessary, though, since kleptoplasty is a complex topic and, especially the kleptoplasty by the species from aphotic anoxic zones is not well understood, yet. Therefore, it is a good idea to discuss, what we know about kleptoplasty (even if it is from aerated environments), before we switch to the things, we don't know, yet. Also I already had some text in the original manuscript, where I stated that at least the intertidal kleptoplastic species, which usually live in oxygenated environments, sometimes are buried in the deeper anoxic layers in the sediment. This is quite common for *Haynesina germanica* but also for some *Elphidium* species. For example, I am working on samples from a hypoxic Canadian Fjord basin at the moment (unpublished data, that is not discussed in this manuscript). The only two species, present in these sediments are *Stainforthia fusiformis* (denitrifier) and *Elphidium albumbilicatum* (no nitrate storage but kleptoplastic). I extended the section about the periodic exposure of intertidal species to  $\text{O}_2$  depleted condition in the revised MS to address this concern by the reviewer:

“Intertidal foraminifera are often exposed to  $\text{O}_2$  depleted or even anoxic conditions, when water stagnates during low tide or if they are transported to deeper anoxic sediment layers by bioturbation (Rybarczyk et al., 1996; Cesbron et al., 2017). Oxygen penetration depths in tidal flats can vary

between a few mm during low tide to several cm during high tide (Jansen et al., 2009). Thus, intertidal foraminifera are often exposed to anoxia, even within the first cm of the sediment column. *H. germanica* is also supposed to occur in black sediments of the British salt marsh tide pools (Bernhard and Bowser, 1999), which likely become anoxic during a tidal cycle (Rybarczyk et al., 1996) and it was among the first recolonizers of a Fjord suffering of organic pollution (Cato et al., 1980; Bernhard and Bowser, 1999)."

To address the concern by the reviewer that LeKieffre et al. (2018) and Jauffrais et al. (2019) did not incubate under anoxia, I adapted these sections slightly. For the LeKieffre study, I added that it has been done under aerated conditions:

"Recently, LeKieffre et al. (2018) showed in **(aerated)** incubation experiments with  $\text{H}^{13}\text{CO}_3^-$  and  $^{15}\text{NH}_4^+$  during a light/dark cycle that *Haynesina germanica* is indeed able to fix inorganic carbon and nitrogen under light exposure."

Regarding Jauffrais et al. (2019), I intended to cite their discussion, where they speculate that kleptoplasty in photic environments might also be an adaptation to periodic  $\text{O}_2$  depletion. They have a whole paragraph about this hypothesis in the discussion of their paper. I did not want to discuss their (aerated) experiment in my sentence. To make this clear I adapted the sentence in the revised manuscript accordingly:

"Kleptoplasty might thus be an additional adaptation of foraminifera from photic environments to stay active during periods of  $\text{O}_2$  depletion, **which already has been hypothesized by Cesbron et al., 2017.**"

**Reviewer:** The paragraph about kleptoplastidic foraminifers from aphotic anoxic zones are relevant to this Review as two species inhabit anoxic conditions: *Nonionella stella* from Santa Barbara Basin and *Virgulina fragilis* from a few habitats (Venezuela, Japan, Namibia; unfortunately the New Zealand population has become decimated as eutrophication remediation has progressed). It is surprising that *V. fragilis* has not been discussed at all in this Review.

**Reply:** *Virgulina fragilis* is now immediately mentioned in the first sentence of this paragraph:

"Less well understood is the phenomenon of kleptoplasty, observed in the benthic foraminifers *Nonionella stella*, *Virgulina fragilis* and *Nonionellina labradorica* that can thrive below the photic zone and often inhabit  $\text{O}_2$  -depleted sediments (Cedhagen, 1991; Bernhard and Bowser, 1999; Grzymski et al., 2002; Bernhard, 2003; Tsuchiya et al., 2015; Jauffrais et al., 2019; Gomaa et al., 2021; Powers et al., 2022)."

I don't see, why eutrophication remediation is a bad thing.

**Reviewer:** Why is Figure 2 a figure? It is merely an equation and should be included as such.

**Reply:** Figure 2 is now included into the paper as an equation and not as figure anymore.

**Reviewer:** Figure 3 shows a common biogeochemical pathway, denitrification (do a Google search!)—why include it here as a figure? Further, given Orsi et al. and Gomaa et al. also documented this pathway for other foraminifera communities/species, why are these not cited as well as the Woehle et al. papers? If this figure is similar to a previously published image, copyright permission may be required.

**Reply:** Figure 3 not only shows the common denitrification pathway but also includes the alternate oxygenic pathway that might be catalyzed by the nitric oxide dismutase (Nod). Since it is a biogeochemical pathway, as the reviewer states in the first sentence, it cannot be copyrighted. I actually don't even know a publication with a similar depiction of this pathway. The image in the original figure by Woehle & Roy et al. (2018) is completely different and neither Orsi et al. (2020) or Goma et al. (2021) use a similar depiction. Woehle & Roy et al. (2018) was mainly cited here since it was the first paper that described the possible alternative oxygenic denitrification. I have no objections against adding Orsi et al. (2020) and Goma et al. (2021) that later confirmed the presence of these enzymes and will cite them in this figure, too.

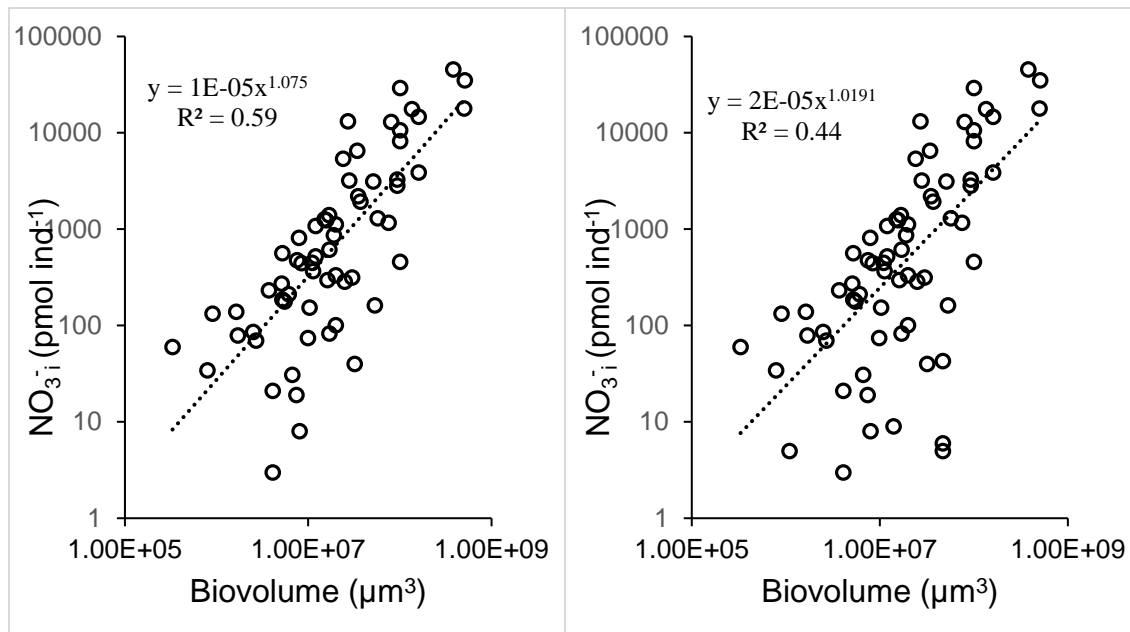
**Reviewer:** The point of Fig. 6 is not clear. For example, bacterivory by benthic foraminifera was established in the 1960s (work of JJ Lee and colleagues), so this is nothing new. Foraminiferal preying on metazoans also is not new—see, e.g., Alan Be papers (1980s), Bowser on carnivory by Antarctic foraminifera (1990s), etc. If insist on only citing Dupuy then must add “e.g.,” as it is only an example. Term “boliviniids” should be capitalized without italics; same for “globobuliminids”.

**Reply:** The whole “ecology” section has been rewritten. According to the constructive feedback by another reviewer (Andrew Gooday). The name of the section has been changed to “Trophic interactions in O<sub>2</sub> depleted environments”. For the detailed changes in the text of this section see response letter to Andrew Gooday. Also fig.6 has been adapted to the new text in this section. It will provide schematic depictions of the different trophic strategies of foraminifera from O<sub>2</sub> depleted environments such as selective herbivory, seasonal herbivory and detrivory. The depiction of phagocytosis will be kept in this figure, because it is important for all these strategies and certainly present in anoxia, as shown by Orsi et al., 2020. I will swap the text “prey” with “food” to clarify, that not only prey organisms can be vacuolized. I am not completely opposed to remove the depiction of the foraminifer preying on nematodes but I would prefer to keep it for the sake of completeness.

**Reviewer:** Fig. 7 presents a subset of data, only those that fit some threshold. The important question is What does this regression look like when including the data that was excluded?? Remove one of the “from” in “from from” of the caption. Figure captions are generally far too long.

**Reply:** The threshold was chosen to exclude foraminifera that do not store nitrate in their cells. In my opinion it does not make too much sense to include species that don't store nitrate to show the correlation between cell volume and nitrate storage in foraminifera that store nitrate. Nevertheless, only a total of six datapoints have been excluded in the original regression. Most of the time there is no cell volume given in literature for species with zero nitrate storage (see for example Pina Ochoa et al., 2010; PNAS). Nevertheless, I did an additional regression, where I included the 6 datapoints that fell below the threshold (<1 mM). All these datapoints included foraminifera with a nitrate content of less than 10 pmol/cell, which is very low and in my opinion not related to nitrate storage for denitrification. The regression itself is still significant and looks very similar but the R<sup>2</sup> is a bit lower. A comparison of both regressions is shown below. I would prefer to keep the original figure in the manuscript. Figure captions, especially the one concerning the microhabitats are generally shortened now.





Above: Left: Original figure that excluded 6 data-points, which fell below the threshold (<1 mM). Right: Regression that includes all available datapoints, including foraminifera with intracellular nitrate concentrations of < 1 mM.

**Grammatical and other minor specifics:**

The first person singular (“I”) is used in many places (e.g., lines 43, 46, 51, 52), which is unconventional for scientific literature, which typically is in the third person plural.

**Reviewer:** Line 11: “artificial fertilizer” is a peculiar phrase. Most would say “eutrophication”.

**Reply:** Done.

**Reviewer:** Line 15 notes that certain foraminifera are “unique amongst eukaryotes” without elaboration of details. Again, this is an overstatement without supporting details. Such phraseology must be used with caution.

**Reply:** Good point. “unique” has been changed to “rare”.

**Reviewer:** What is “heterotrophic denitrification”? First use: line 17.

**Reply:** Heterotrophic denitrification is described in detail within the text and even in two figures (2&3). Line 17 is within the abstract. The abstract would be a bit too long, of every used term would be explained in detail. Otherwise I would also have to explain terms like “anaerobic metabolism”, “kleptoplasty”, “dormancy” or “phagocytosis” that are all mentioned in the abstract.

**Reviewer:** Line 34 would read better if it was changed to “Nevertheless, much has changed in our perspective about...” (less “wordy”).

**Reply:** Another reviewer suggested to change this sentence as well and merge it with the next sentence to assure that this part is not too wordy. This sentence has been changed to:

“Nevertheless, advances in methods to analyze the metabolic rates, intracellular nitrate storage and molecular genetics of foraminifera have changed our understanding of strategies such as anaerobic metabolism that help them to withstand O<sub>2</sub> depletion.”

**Reviewer:** Line 50 equates organic matter to food. This is an arguable point as organic matter may be refractory, etc and otherwise not necessarily “food”.

**Reply:** This is a good point. To avoid any misunderstanding, I changed the sentence to:

“...non refractory organic matter that can be used as food.”

**Reviewer:** Line 51 notes that there is scarce knowledge of the ecological interactions of benthic foraminifera from low oxygen to anoxic habitats. This, simply, is not true. Indeed, in Section 3, (lines 285-310) the authors notes that there is much to review on the ecology (although this discussion is about trophics).

**Reply:** The whole “ecology” section has been rewritten. According to the constructive feedback by another reviewer (Andrew Gooday). The name of the section has been changed to “Trophic interactions in O<sub>2</sub> depleted environments”. For the detailed changes in the text of this section see response letter to Andrew Gooday.

**Reviewer:** Line 52 would read better as “...role of foraminifera in marine biogeochemical...”

**Reply:** Done.

**Reviewer:** The statement spanning lines 58-59 is redundant with the section header, so it should be omitted.

**Reply:** Done. This statement has been deleted.

**Reviewer:** Line 61 would be better as “metabolism of benthic foraminifera.”

**Reply:** According to the suggestion of another reviewer this sentence has been deleted.

**Reviewer:** Line 76 mentioned gromiids, which are not foraminifera. If discussion of this group remains, the title much include this taxon name.

**Reply:** See my answer from above:

“I am not opposed to add the “*Gromia*” as well but the title is already quite long and gromiids are only a minor part of this review. Nevertheless, I clarified now specifically in the text that gromiids aren’t foraminifera, when gromiids are first mentioned in the text:

“Other studies showed that bacterial endobionts likely perform denitrification in some allogromiid foraminifera and gromiid species (Bernhard et al., 2012a; Høglund et al., 2017). Gromiida are a separate group of protists within the Rhizaria and closely related to foraminifera.”

In addition, I separated foraminifera and gromiids in table 2.”

**Reviewer:** Line 77 states the presence or size of the intracellular nitrate, but nitrate cannot be “sized”. Better alternatives include concentration, amount or magnitude.

**Reply:** Changed “size” to “magnitude”.

**Reviewer:** Line 92 cites Bernhard et al. 2012a when Bernhard et al. 2012b should be cited.

**Reply:** Changed accordingly.

**Reviewer:** Line 94 incorrectly infers that bacteria are “killed” by antibiotics, when in reality bacterial activities are inhibited when exposed to the appropriate antibiotics.

**Reply:** This sentence has been changed accordingly:

“...when the activity of potential bacterial symbionts would be inhibited.”

**Reviewer:** Line 108 states that genomes were obtained by Gomaa et al. (2021) but this is not the case and may not be the case in Orsi et al. (2020) either.

**Reply:** This is right. “genomes” has been changed to “transcriptomes”.

**Reviewer:** Why is a publication listed with two author names plus “et al.” (line 113)? There is no need of this given there are no other 2022 papers with Woehle and Roy as the first two authors.

**Reply:** Both papers Woehle & Roy et al. 2018 & 2022 were papers with shared first authorships. I wanted to do the “second-first author” justice, because otherwise, mainly the first name will be shown as citation in other papers. Unfortunately, this was not uniform along my manuscript, due to the use of a reference manager. I corrected this and now use “Woehle & Roy et al.” all along the manuscript.

**Reviewer:** Line 122 requires a literature citation(s) for the sentence ending “...strong reactivity”.

**Reply:** According to another reviewer (Frans Jorissen) the text was going to fast over some statements and he already criticized that I have to extend the statement about the oxygen reactivity. Now this part is longer and more citations have been added:

“Larger amounts of O<sub>2</sub> might supply this demand but also harm the cell. For example, O<sub>2</sub> can inhibit the growth of some obligate anaerobes poison enzymes that are important for their metabolism (Lu and Imlay, 2021). Also for aerobes O<sub>2</sub> can be harmful. “Hyperoxia”, an excess supply of O<sub>2</sub>, leads to damaging effects by highly-reactive metabolic products of O<sub>2</sub> (free O<sub>2</sub> radicals) that inactivate enzymes in the cell, damage DNA and destroy lipid membranes (Frank and Massaro, 1980). Furthermore, foraminifera can store NO<sub>3</sub><sup>-</sup> within vacuoles, due to its lower reactivity and still have an electron acceptor reservoir if NO<sub>3</sub><sup>-</sup> is depleted in their microhabitat. This is not possible for O<sub>2</sub> due to its high reactivity (Auten and Davis, 2009).”

**Reviewer:** Line 133 should remove “denitrifying” as that word appears later in the sentence.

**Reply:** Done.

**Reviewer:** Line 139 should cite the original literature that documented “epifauna, shallow infauna, and deep infauna” foraminifera (Bruce Corliss *Nature* 1985).

**Reply:** I added the following sentence to this section:

“The presence of this species specific microhabitat structure has first been documented by Corliss (1985).”

**Reviewer:** Line 146 and elsewhere, the use of the verb “prefer” (or preference) should be avoided as foraminifers do not have conscious thought.

**Reply:** This is a rather philosophical debate, if a “preference” prerequisites a conscious thought. We all agree that certain foraminifera species have certain food or microhabitat preferences. Some species selectively ingest phytodetritus, if available. If fresh phytodetritus is not available they ingest other food particles. Isn't this a preference for phytodetritus? Nevertheless, in the revised version of the manuscript, I tried to reduce the usage of “prefer” or “preference”.

**Reviewer:** On line 150, the species of *Globobulimina* that are considered to be deep infaunal / facultative aerobes are not listed.

**Reply:** Added “spp.” after *Globobulimina*.

**Reviewer:** Use of “basically” on line 151 is colloquial and superfluous; the term should be omitted.

**Reply:** “basically” has been deleted.

**Reviewer:** The phrase “competitive stress” (line 153) is unconventional. Perhaps the intention was “competitiveness”?

**Reply:** Changed “competitive” to “competitiveness”.

**Reviewer:** The references are often placed at the end of a sentence when the papers do not all show the same thing. This approach should be avoided. For example, the sentence spanning lines 155-158 should have Schmiedl and Mackensen (2006) cited after “shallow infaunal lifestyle”, not at the end as that paper did not assess denitrification or nitrate storage.

**Reply:** I divided the references in this sentence and moved Schmiedl and Mackensen (2006) as suggested by the reviewer.

**Reviewer:** The statement on line 159 is awkward because “used” can mean “utilized”, but it seems the intent here is more like “accustomed”. A suggested edit is “that typically occur at the sediment-water interface or on elevated surfaces.”

**Reply:** Edited and changed as the reviewer suggested.

**Reviewer:** Line 164 should read “spp. may denitrify under”.

Line 167 should read “...if they must, due to their...”

Line 170 should read “research still continues...”

**Reply:** All done.

**Reviewer:** Throughout, “chapters” should be called “sections”.

**Reply:** Changed “chapter” to “section” throughout the MS.

**Reviewer:** The sentence spanning lines 169-171 is poorly worded as published papers have already determined denitrification rates, meaning the wording cannot include any literature citations.

**Reply:** This sentence has been changed, according to suggestions of another reviewer: “Research to measure denitrification rates in different benthic foraminiferal species continues (Langlet et al., 2020; Choquel et al., 2021). This will add to the scarce available data and contribute to estimates of the role of foraminifera in oceanic N-cycling

**Reviewer:** The order of subsections in Section 2.1 is illogical as it starts discussing an active process (denitrification), then discusses a inactive process (dormancy), and then discusses an active process again (kleptoplasty).

**Reply:** The order was supposed to be alphabetical (foraminiferal) “denitrification”, “dormancy”, “kleptoplasty” and “other recent developments...”. It was not supposed to be ordered after active and inactive processes. I changed the first header to “denitrification by foraminifera” to make the alphabetical order more accessible.

**Reviewer:** Line 183 should read “well-aerated condition. They interpreted this...” (two changes)

**Reply:** Done.

**Reviewer:** The paragraph spanning lines 189 to 200 consistently italicizes “sp.”, which is incorrect. This should never be italicized. Line 201 italicizes “spp.” incorrectly.

**Reply:** Done.

**Reviewer:** Line 197 should read “...acids, which was not the case...”

**Reply:** Done.

**Reviewer:** It is not clear why paragraph spanning lines 201-209 is included in the dormancy section. If there is a connection to dormancy, this must be explicitly stated.

**Reply:** This paragraph dealt with ultrastructural changes of *Ammonia* sp. exposed to anoxia. A survival strategy by *Ammonia* sp. under exposition to anoxia is dormancy, as discussed in other parts of this section. So some of the documented ultrastructural changes might be related to dormancy. I apologize that this was not clear enough in this paragraph. To address this, I state this directly now in one of the sentences:

“These were interpreted as endobionts but might also be parasites that could not be fended off, due to the drastically reduced metabolism during dormancy under anoxia.”

**Reviewer:** Line 206 should read “...could not be fended off” (although this is a rather colloquial statement). Further, this statement should cite the original publication that showed the transition from endobionts to parasites to necrophagy (i.e., Bernhard et al. 2010 *Paleoceanography*).

**Reply:** Done. Added the following sentence:

“A continuum of intracellular bacteria including prey in food vacuoles, endobionts, parasites and necrophages has been documented before in benthic foraminifera from cold seeps (Bernhard et al., 2010). It already has been hypothesized by the authors that bacteria switched their function from endobionts to predators, depending on the vitality of the host cell.”

**Reviewer:** Line 214 should read “...this research originated in the 1970’s (Lopez...”

**Reply:** Done.

**Reviewer:** Line 232 must spell the genera name properly: *Nonionella*, for *N. stella*. The genus for *N. labradorica* is *Nonionellina* (not the same as *Nonionella*). Further, Powers et al. (2022) *Frontiers in Mar. Sci Sect on Mar Microbiol and Ecol.* should be cited in the string of citations on line 234.

**Reply:** Corrected the typos in the genera name and added the citation.

**Reviewer:** The spelling of Grymsky on line 241 must be corrected (it is Grzymiski).

**Reply:** Done.

**Reviewer:** Line 242 should read "...calculated that the required amount..."

**Reply:** Done.

**Reviewer:** Line 248 should read "... Gomaa et al. (2021) support observations of Grzymiski et al (2002)."

**Reply:** Done.

**Reviewer:** It is surprising that Powers et al. (2022 *Frontiers*) is not discussed at all in this Review. '

**Reply:** This paper is now discussed in detail in the revised manuscript:

"Most foraminifera species from O<sub>2</sub> depleted habitats possess numerous peroxisomes that are usually associated with mitochondria and the endoplasmic reticulum (Bernhard and Bowser, 2008). Bernhard and Bowser (2008) hypothesized that these peroxisome proliferations might be used to either metabolize H<sub>2</sub>O<sub>2</sub> and other highly reactive oxygen species that are produced within the chemocline close to the oxic/anoxic boundary or to reduce the oxidative stress by these compounds. Indeed, they showed in an experiment that ATP concentrations in foraminifera increased proportional to ambient H<sub>2</sub>O<sub>2</sub> concentrations. A recent study on transcriptome and metatranscriptome of *N. stella* and *B. argentea* from the Santa Barbara Basin revealed that these species utilize an adaptable mitochondrial and peroxisomal metabolism, depending on the chemical treatment in the experiment (Powers et al., 2022). The high plasticity of their peroxisomal and mitochondrial metabolism might be substantial for survival at the highly variable conditions at the chemocline in the sediments. The results by Powers et al. (2022) indicate that at least some processes that are involved in foraminiferal denitrification are associated with mitochondria. Interestingly, the expression of denitrification related genes in both species was upregulated after incubation with elevated H<sub>2</sub>O<sub>2</sub> but without NO<sub>3</sub><sup>-</sup> and downregulated, if they were incubated without H<sub>2</sub>O<sub>2</sub> but with NO<sub>3</sub><sup>-</sup>, compared to a control treatment with both H<sub>2</sub>O<sub>2</sub> and NO<sub>3</sub><sup>-</sup>. In the same way several peroxisomal processes were upregulated in the H<sub>2</sub>O<sub>2</sub> only treatment. In addition, despite that both species are able to denitrify, Powers et al. (2022) found distinct metabolic adaptations to anoxia in both species. For example, a quinol:fumarate oxidoreductase, which is considered as an adaptive mechanism for anaerobic respiration in eukaryotic organisms, was present in *N. stella* but not in *B. argentea*. Vice versa, *B. argentea* has the capacity to digest food vacuole contents under O<sub>2</sub> depletion, while *N. stella* was lacking food vacuoles (Powers et al., 2022)."

**Reviewer:** Line 261 should write out the genus name for *B. argentea* (Bolivina).

**Reply:** Done.

**Reviewer:** Line 268 should read "...observation was made by..."

**Reply:** Done.

**Reviewer:** For line 270, the term "presumably" should be inserted to read "Presumably living foraminifera..."

**Reply:** Done.

**Reviewer:** Line 276 "Though this requires...energy." is not a sentence. Please rewrite.

**Reply:** This part has been rewritten, due to the suggestion of another reviewer:

"These processes (calcification and the ingestion of prey cells by phagocytosis) require bursts of high energy, which the authors suggest is generated by dephosphorylation of an intracellular creatine phosphate storage to regenerate ATP from ADP."

**Reviewer:** Please correct spelling of "metatranskriptome" on line 279.

**Reply:** Done.

**Reviewer:** Line 281 should read "...found evidence of another anaerobic metabolism..." (as denitrification is an anaerobic metabolism).

**Reply:** Done.

**Reviewer:** Line 282-283 should include Gomaa et al. (2021) as these processes were also documented.

**Reply:** Changed this part to: "Orsi et al. (2020) and Gomaa et al. 2021 also found evidence for another anaerobic metabolism in foraminifera from the Namibian shelf. Their data indicates that the foraminifers metabolize hydrolyzed organics to produce ATP using fermentation and fumarate reduction."

**Reviewer:** Line 287 discusses a study with 60  $\mu\text{M}$   $\text{O}_2$ , which is very high from many "hypoxia" perspectives.

**Reply:** I am a bit confused, since hypoxia are not mentioned in this context. I only mentioned "oxygen depleted environments" in a sentence before and I guess we can agree that "less than 60  $\mu\text{M}$ " in the bottom water can be considered as oxygen depleted. In addition, the oxygen penetration depth into the sediments at this location is very low. I added a statement about the  $\text{O}_2$  penetration depth at this location and also stated that many of these observations, especially regarding shallow infauna also apply for foraminifera from well oxygenated environments.

**Reviewer:** Line 292 should read "cancellata and Chilostomella".

**Reply:** Done.

**Reviewer:** Consider using the typical term for deposit feeders (detritivore) on line 293.

**Reply:** Done.

**Reviewer:** Line 294 must include “e.g.,” in the beginning of the citations as there are additional studies of carnivory by foraminifers.

**Reply:** Done.

**Reviewer:** Line 298 uses the term “thrive” (used earlier in the manuscript too) yet there is no documentation that this species is truly abundant in the setting being discussed.

**Reply:** *Globobulimina auriculata* is relatively abundant in the oxygen depleted Alsbäck Deep within the Gullmar Fjord. It shares the habitat with the closely related *Globobulimina turgida*. Both species have a very similar appearance and have thus been lumped in some early studies about foraminifera assemblages in the Gullmar Fjord. Now we have seen the differences between both species in the transcriptome (Woehle & Roy et al., 2018), in their feeding strategies (Glock et al. 2019) and in subtle morphological features, such as appearance and color of the cytoplasm and the shape of the aperture. Nevertheless, since no study is published, yet, about the abundances of both species, I decided to change this sentence accordingly. I avoided “thrives” and wrote:

“The species *G. auriculata* denitrifies and lives under oxygen depleted conditions (Woehle & Roy et al., 2018).”

**Reviewer:** “Predator prey” must be hyphenated on line 310.

**Reply:** Done.

**Reviewer:** The sentence spanning lines 312-313 should be omitted as it is highly redundant.

The use of “not exotic” on line 314 requires explanation. Exotic to what?

**Reply:** Done. Both sentences have been deleted, due to being redundant.

**Reviewer:** Line 316 should use either “established” or “suggested” instead of “pointed out” which is colloquial. Consider replacing one of the “some” on line 317 (“In some environments, such as some habitats...” (possible replacements are “certain”, “selected”).

**Reply:** Done.

**Reviewer:** It would be good to add other publications on line 318 that have calculated the contribution of foraminifera to total denitrification such as Choquel et al., Glud et al., etc. While it is understood that the author is promoting his publications, it gets a bit much sometimes.

**Reply:** Done. I added both references. Glud et al. showed that foraminifera have only a minor contribution to the nitrogen cycle at Sagami Bay but they also showed that it is significant. So I guess this paper can be cited here. I apologize that I forgot to cite Choquel et al. 2021. It was not my intention to only promote my own publications.

**Reviewer:** “keyplayers” is two words (line 318).

**Reply:** Done.



**Reviewer:** The text spanning lines 327 to 330 should explain why diagenetic models are being discussed. Most people will be interested in biogeochemical modeling vs diagenetic models.

**Reply:** I don't really understand the statement regarding biogeochemical modeling vs diagenetic modeling. I mean, if redox processes that happen in the upper sediment column during early diagenesis are modelled, using a diagenetic model, this is also biogeochemical modeling, right? Why should we make a difference here. The "diagenetic model" is mainly discussed, because the model used by Dale et al. models early diagenesis by including biological nitrate transport, instead of just using a diffusion-reaction model. To assure that the "diagenetic model" part regards the Dale et al. study I added "other" before diagenetic models:

"The NO<sub>3</sub><sup>-</sup> storage in denitrifying foraminifera, but also in some sulfur bacteria, such as *Beggiatoa*, is of greater importance for benthic biogeochemical cycling, due to the potential of biological transport of these intracellular reservoirs (Dale et al., 2016). Most of the **other** diagenetic models that describe and calculate benthic N-cycling..."

**Reviewer:** Line 337: change capitalized "D" in "Data" to lower case.

**Reply:** Done.

**Reviewer:** The observation that foraminifera have served as a nucleating site for phosphorites (lines 348 to 350) does not necessarily indicate their active use of precursors as there is known presence of other mineral associations in foraminifer tests (e.g., pyrite framboids).

**Reply:** This section might indeed have been a bit too short and some important information was missing. There have been several studies that showed that the large intracellular polyphosphate enrichments in sulfur bacteria can facilitate phosphorite formation at the upper boundary of the Peruvian OMZ that is rich in bacterial mats and phosphorite deposits. The phosphorite deposits at the lower boundary of the Peruvian OMZ are different, because these bacterial mats are usually not present and the phosphorite grains have a similar size and shape of foraminifera. These phosphorite grains are also abundant in the surface fraction of the sediments. In the Peruvian OMZ, living foraminifera abundances are very high. The sediments are thus a mixture of living forams, phosphorite grains with a coarse shape and size of a foram and every intermediate step in between. It is likely that their high intracellular phosphate storage, together with the calcium storage to precipitate their tests results in a supersaturated apatite microenvironment within their shells and initiates apatite formation. This has been also suggested for other organisms before. All this is discussed in detail in my 2020 GCA paper ("A hidden sedimentary phosphate pool inside benthic foraminifera from the Peruvian upwelling region might nucleate phosphogenesis"). To address these issues and avoid further misunderstandings, I extended the text in this section:

"In addition, there is evidence that the intracellular phosphate storage in foraminifera facilitates phosphogenesis in some environments, similar to the intracellular polyphosphate enrichments in some sulfur bacteria (Schulz and Schulz, 2005). The release of phosphate after breakdown of these polyphosphates to harvest energy in times of electron acceptor depletion results in apatite supersaturation and initiates phosphogenesis (Schulz and Schulz, 2005). Sediments at the lower boundary of the Peruvian OMZ contain many small phosphorite grains with similar size and shape of foraminifera (Manheim et al., 1975; Glock et al., 2020). The sand fraction of the surface sediments in this region is a mixture of pristine living foraminifer shells with dead tests that show a transition from shells that are filled with phosphorites until small phosphorite grains that only retain the size and coarse shape of a foraminifer. It is likely that a post mortem release of the intracellular phosphate storage results in a supersaturated microenvironment within the shells that initiates apatite

formation (Glock et al., 2020) in a similar way as it has been suggested for other organisms (Kulakovskaya, 2014).”

**Reviewer:** The single sentence spanning lines 356 to 361 must be broken into two or three sentences.

**Reply:** Done. Divided into two sentences.

**Reviewer:** In section 4.1, the author does not address the very large variability of vacuole volume in cytoplasm from varied foraminifers (see Fig 2 in LeKieffre et a 2018 Mar Micropaleo).

**Reply:** I don't think the vacuole volume has too much to do with the intracellular nutrient storage but rather the nitrate concentration in the vacuoles. Vacuoles are also present in foraminifera that do not store nitrate. In addition, vacuoles can have a variety of functions and most are not related to nitrate storage. For example: Some vacuoles are related to calcification and others to digestions. In addition, not all structures that are considered as vacuoles are indeed seawater vacuoles but vesicles with various functions. For example, many of the small vesicles that are observed with TEM in for example *Ammonia* spp. are indeed acidocalcisomes that lost their content during preparation for TEM.

**Reviewer:** The Section on paleoceanography (Sect 4) needs to be shortened as an “in prep” manuscript (Hoogakker et al.) is cited in most sentences (appears at least 5 times over ~75 lines). Such passages are irritating—just leave that information to the Hoogakker team to present. Further, most of this discussion is about environments with oxygen, which is not anoxia, impacting the decision to change the title or focus of the Review, as discussed earlier in this critique.

**Reply:** The paleo-part will be deleted and only briefly addressed in the introduction. So many of the remarks below (from line 399 to 450) will not be relevant anymore and not being replied in detail.

**Reviewer:** Do not italicize family names (line 399) and do not italicize “spp.” (line 397 and as noted earlier).

Line 399 is not a sentence. Please rewrite.

The format “*C. spp.*” is not allowed (line 416). Here, the genus name must be fully written.

Line 424: is 11 years still considered “recent”?

Aside from citing Erez, newer references should be cited regarding foraminiferal calcification mechanisms (e.g., works by Toyofuku and/or de Nooijer).

The list of 22 publications in one sentence (lines 432-435) is excessive and not the approach used in other places earlier in the manuscript.

Statement on line 439 should read “This offset is referred to as...” because that notation was used in the McCorkle paper from decades ago—the terminology is not new. Line 441 should cite McCorkle papers also, not solely the new publications.

“Height difference” (line 442) is a peculiar way to discuss what I believe the author intends, which is depth of calcification.

Line 445 should present values for “lower [O<sub>2</sub>] range”.

Line 454 should read "...this index are ongoing , with recent developments... Tetard et al. (2021) and Kranner et al. (2022)." (five changes)

Line 450 uses the phrase "appears obvious" which is rather rude. Hindsight is 20-20 vision, correct? Meaning of course things seem obvious now but no one / few thought foraminifera could be anaerobes back then. The author is urged to rewrite this.

**Reply:** As mentioned above, the paleo-part will be deleted and only briefly be addressed in the introduction. So many of the remarks above (from line 399 to 450) will not be relevant anymore and not being replied in detail.

**Reviewer:** Line 464 should be singular as there is only one author.

**Reply:** Done.

**Reviewer:** The proper way to cite one's grant in Acks (line 467 is "... (DFG) through Heisenberg grant GL 999/3-1 to N.G." (Initials can be used if the grant recipient is an author of the manuscript).

**Reply:** Done.

**Reviewer:** Species names must be italicized in the cited literature section.

Journal name is missing from van Dijk et al. 2019 (and most would alphabetize that under V). Same for de Frietas (should appear in the D's).

Proper citation for Glock et al 2012b is:

Glock, N., Schönfeld, J., Mallon, J. (2012). The Functionality of Pores in Benthic Foraminifera in View of Bottom Water Oxygenation: A Review. In: Altenbach, A., Bernhard, J., Seckbach, J. (eds) Anoxia. Cellular Origin, Life in Extreme Habitats and Astrobiology, vol 21. Springer, Dordrecht.  
[https://doi.org/10.1007/978-94-007-1896-8\\_28](https://doi.org/10.1007/978-94-007-1896-8_28)

Line 583: do not capitalize "auriculata"

Why is Glock et al. (2019) PNAS repeated twice in the Literature cited section (2019a and 2019b)?

The citation for Grzymiski et al. (2002) has added seven extraneous authors. Why? The author list is Grzymiski, Schofield, Falkowski and Bernhard.

The "o" in Jorgensen has a slash through it (Scandinavian letter).

Line 849 should read "pH", not "PH".

**Reply:** I thank the reviewer for this thorough check of the reference list. All references will be corrected carefully in the revised manuscript.

**Reviewer:** Names in Table 1 must be alphabetized. Terms must be defined ("specific denitrification" is truly a rate presented on a per volume basis).

**Reply:** Done. Terms are defined as follows: "Individual denitrification rates refer to average rates per individual while specific denitrification rates refer to rates normalized to the biovolume of the foraminifers."

**Reviewer:** Table 2 should remove gromiida unless the title changes. And, proper: Gromiida, or “gromids”

**Reply:** Done.

**Reviewer:** Italicize ‘H’ in Haynesina in Table 3. “Labrospira cf. subglobosa” should read “*Labrospira* cf *L. subglobosa*” (add first letter of genus name italicized and without italics for “cf”).

**Reply:** Done.