Dear Editor,
We are grateful for the constructive and insightful reviews of our manuscript "Effects of photosymbiosis and related processes on planktic foraminifera-bound nitrogen isotopes in South Atlantic sediments", and for the opportunity we were given to revise the manuscript and respond to questions and comments.
We carefully considered the comments and suggestions of both reviewers, which improved the manuscript, and we give detailed responses to their comments in the response to reviews. In addition, we added a few editorial changes to improve readability of the text.
In the response to reviews, **black** text is the original comment from reviewers, and **green** text is the associated response from the authors.
On behalf of all the authors,
Alexandra Auderset

Reviewer 2 (anonymous)
General comments:

The manuscript entitled "Effects of photosymbiosis and related processes on planktic foraminifera-bound nitrogen isotopes in South Atlantic sediments" by Auderset et al. reported the species-specific FB-$\delta^{15}N$ from sediment core samples together with test $\delta^{13}C$ and $\delta^{18}O$, which is important to validate the utility of FB-$\delta^{15}N$ to detect fossil foraminiferal photosymbiosis. They found consistently lower FB-$\delta^{15}N$ on dinoflagellate-bearing foraminifera than non-dinoflagellate/non-symbiotic species. They also discussed the offset between the dinoflagellate-bearing species' FB-$\delta^{15}N$ and others, especially an exceptionally high offset in DSDP Site 516 compared to the global compilation of FB-$\delta^{15}N$. They proposed the possible influence of regional differences in nitrate $\delta^{15}N$. This study is important to gain our understanding of the FB-$\delta^{15}N$ proxy as a tool to detect fossil photosymbiosis, and as a tool to reconstruct past N cycling. This study presents invaluable information on FB-$\delta^{15}N$, which will fuel future studies in this field.
The manuscript is overall well-written, and carefully discussed with adequate data sets. However, some statistical representations seem incorrect, and some discussions need to be reformulated. The paper would be more improved if the following points are fully considered.

Specific comments:

1. Size-specific $\delta^{13}C$
I would recommend not to use R^2 as a metric of the strength of relationships. In the first place, R^2 is not a "regression coefficient (L206)" but a "coefficient of determination", a measure of the proportion of the variance in the dependent variable that is explained by the independent variables in the regression model (goodness-of-fit). Regression coefficient is a slope in a linear regression model. I assume the authors intended to say "correlation coefficient (normally denoted by *r*)" in this sentence. Please report the *r* and *p*-value together in Table S1.
Replaced R^2 with "r-value" in the text and added r- and p-values to the table.

In addition, R^2 for *G. bulloides* (R^2 =1.00) is meaningless since it is the result of two-point linear regression (n=2). It can be omitted or the sample size should be shown in the same table.
Removed R^2=1.00 and replace it with n/a.

I would like to confirm the largest size class of each species. 400um is large enough for *G. ruber* but may be still in the juvenile stage for species like *G. siphonifera* which often reaches 800um in maximum length. For example, Bornemann and Norris (2007) measured size-specific $\delta^{13}C$ of modern species, and they used 13 size fractions ranging from 75um to 800um. Having this in mind, the size range from 250um to 400um (or 425um?) used in this study seems too narrow to detect size trends. I won't argue that more size fraction is needed, but caution needs to be paid in the discussion. Please present the largest size class for each species in the sample so that the readers can determine whether the specimens are juvenile or adult/gametogenic stage.
We found many *T. sacculifer* and a few *G. siphonifera* in the size fraction 400-630um, and few *G.truncatulinoides* in the size fraction >630um. We added a sentence in the methods about this "Largest test sizes for *T. sacculifer* and *G. siphonifera* were observed in the 400-630 µm size fraction, and *G. truncatulinoides* in the >630 µm size fraction." And in the discussion: "The weak/ near-zero slope for *G. siphonifera* $\delta^{13}C$ (Fig. 2Se) could be the result of the size fraction between 125-400 µm used in this study, as we also observed (but did not measure) a small amount of *G. siphonifera* in the fraction between 400-630 µm. Nevertheless, the…" and "That *G. truncatulinoides* falls into the same range of slopes (as well as absolute $\delta^{13}C$ values) as *T. sacculifer* and *G. ruber* and has a steeper slope than *G. siphonifera* indicates either that the size fraction was too narrow and we observe the difference between juveniles and adults in *G. truncatulinoides* or that the previously…"

In terms of *G. siphonifera*'s size-$\delta^{13}C$ trend, an experimental study by Bijma et al. (1998) is helpful to understand the phenomenon. In their study, *G. siphonifera* type II showed steeper slope than type I. Also, they proposed that effective utilization of the host's respired CO_2 by symbionts inside the test may reduce the effect of $\delta^{13}C$ increase of surrounding microenvironmental seawater DIC. This phenomenon is supported by the recent experimental study by Takagi et al. (2022) which focused on symbiont photosynthesis.
We added two sentences about this in Lines 478-482: "Bijma et al. (1998) showed a steeper slope for *G. siphonifera* type II than type I. The study proposed that *G. siphonifera* type I symbionts more effectively use the host's respired CO_2 inside the foraminiferal test, which may have an effect on the surrounding microenvironmental seawater DIC and thus reduces the $\delta^{13}C$ increase of their tests. This phenomenon is supported by the recent experimental study by Takagi et al. (2022) focussing on symbiont photosynthesis."

2. Size-specific $\delta^{18}O$
At L212-L217, the authors describe the size-specific $\delta^{18}O$ trend with the regression parameters like the size-specific $\delta^{13}C$ discussion, but I don't see any clear correlation between test size and $\delta^{18}O$ based on Fig. S3. I think this part should be fully rewritten.

We updated this paragraph and shortened it (Line 236-238): “In contrast to $\delta^{13}C$, the test carbonate $\delta^{18}O$ shows only a weak correlation with test size (Table S3) and the relationship varies downcore and between species (Fig. S3). The only significant relationship is observed for *G.ruber albus* in the combined time slice (p=0.02).”

3. Supplementary discussion

I suggest including supplementary discussion, especially “Influence of depth habitat on carbonate $\delta^{13}C$ and $\delta^{18}O$”, in the main text. The reason why there is very little difference in $\delta^{13}C$ between dinoflagellate-bearing species and non-symbiotic *G. truncatulinoides* is not clear from the current discussion in the main text alone (L271-272), but the depth profile of $\delta^{13}C_{DIC}$gives reasonable interpretation.

Deleted the discussion around depth habitat influence on $\delta^{13}C$ from the supplements and moved it into the main text (Lines 414-419).

4. FB-$\delta^{15}N$ of *G. siphonifera*

In 4.3, the authors discuss the factors explaining the high values of FB-$\delta^{15}N$ of *G. siphonifera* from the viewpoint of symbiont digestion. Please check the ontogenetic dynamics of *G. siphonifera* chlorophyll content in Takagi et al. (2016, Marine Micropaleontology). Based on that study, the peak chlorophyll content of *G. siphonifera* was always observed before the final chamber formation (or even earlier). At the time of final chamber formation, the largest or second-largest chamber consisting of the majority of calcite mass, the chlorophyll content gets very low, which seems to support the authors’ discussion.

Thanks very much for this insight. We added a sentence and the Takagi reference -- see below.

Alternatively, I think there is a possibility that the physiology of algae (or interaction between the host and the symbionts) may differ between dinoflagellate-symbiont and others. According to Uhle et al. (1999), FB-$\delta^{15}N$ can vary tremendously based on the source and pathways of nitrogen within the host-symbiont system (from NO3- diffusion or from recycled NH4+ pool). Uhle et al. (1999) demonstrated the importance of the NH4+ pool for dinoflagellate-symbiosis, but it may not be the case for pelagophytes. If the non-dinoflagellate symbiont can uptake nitrate from environmental seawater enough efficiently, the recycled NH4+ pool may be not so important and the remaining nitrogen is supplied by diets. Although there are many unknowns, physiological differences should exist between dinoflagellate (relatively large in size, ~10um) and pelagophyte (~1-2um) to some extent. In any case, I believe such physiological differences may also affect the FB-$\delta^{15}N$ differences and so should be considered.

Expanded on the previous section, updated text in Lines 512-529:

“Alternatively, interaction between the host and the symbionts, symbiont physiology and rates of symbiont activity may differ between dinoflagellate-symbiont and others. Even at a low chrysophyte growth rate, if the foraminifera are only slowly harvesting organic matter from the symbionts, then the amount of Chl-*a* will be elevated. In this interpretation, the uniquely low FB-$\delta^{15}N$ of the dinoflagellate-bearing species supports higher photosynthetic rates in dinoflagellate symbionts and, thus, a generally more important role for the symbiosis in the dinoflagellate-bearing species (e.g., as in *O. universa*; Lekieffre et al. (2020)). The shallow depth (i.e. high-light) habitats of the dinoflagellate species is fully consistent with this interpretation, as is the

dominance of dinoflagellate symbioses among modern symbiotic corals (Davy et al., 2012). Moreover, depending on the depths of migration, seasonality, and lateral transport of the different foraminifera species as well as the physiology of the endosymbionts, direct nitrate assimilation by the foraminifera host and its symbionts is possible (Uhle et al., 1999).

Finally, the relatively high FB-$\delta^{15}N$ despite the high average Chl-*a* of *G. siphonifera* may reflect temporal variability in the symbiosis. Takagi et al. (2016) find that the chlorophyll content of *G. siphonifera* has declined by time of construction of largest or second-largest chamber, which comprises the majority of calcite mass and thus of the fossil-bound N.".

5. Discussion on lateral transport

The authors discuss the possibility of lateral transport of *G. bulloides* and *G. siphonifera* from outside of the gyre based on the nitrate $\delta^{15}N$ profile of the North Atlantic. First, I would like to know whether the difference in $\delta^{15}N$ between the inside and outside of such a gyre can be generalized. The example the author showed is of the North Atlantic, with $\delta^{15}N$ difference of 2–3‰. Is this the only example that can be referred to? If the authors want to assume the same mechanism in the DSDP Site 516, I believe at least multiple examples (examples of $\delta^{15}N$ difference between inside and outside gyre, regardless of the region) are needed. Please be very careful when applying a specific phenomenon to your case. Without adequate generalization, discussion sounds opportunistic.

Unfortunately, so far there are no other suitable $\delta^{15}N$ data for the southeast Atlantic and data from the Indian/Pacific Ocean won't be as representative because the influence of N-fixation might be less compared to the Atlantic, and/or overwhelmed by the strong denitrification signals in the tropical/subtropical Pacific. However, nitrate data from the subtropical Indian Ocean by Harms et al. (2019) and Marshall et al. (2023) show a similar nitrate $\delta^{15}N$ gradient than the North Atlantic with ~2.5‰ lower $\delta^{15}N$ of upper-ocean nitrate inside the gyre *vs.* outside. They also report N* values indicating excess N vs P, and thus N_2 fixation in the gyre. Similar effects are observed in the North and South Pacific (Yoshikawa et al., 2018; Yoshikawa et al., 2015; Marconi et al., 2024). We added a sentence on line 618: "A similar upper-ocean nitrate $\delta^{15}N$ gradient is observed in other subtropical gyres in the Indian and Pacific Ocean with ~2.5‰ lower $\delta^{15}N$ inside the gyre *vs*. outside (Harms et al., 2019; Yoshikawa et al., 2015; Yoshikawa et al., 2018; Marconi et al., 2024; Marshall et al., 2023)."

Next, I wonder if it is possible to transport specific species. If their lateral transport hypothesis is true, why not for the other species? Lateral transport is a physical process, so I imagine that there should be no selectivity if they share the same habitat. In addition, I wonder if the amount of laterally transported specimens can exceed over the local population. Based on the authors' argument, I understand that they assume most of the specimens of *G. bulloides* and *G. siphonifera* are from outside of the gyre (2–3‰ difference is directly reflected in the foraminifera). Unless the amount of the transported specimens is large enough, a mixture of the local population and transported specimens makes the resultant FB-$\delta^{15}N$ deviation more subtle. In my impression, discussing lateral transport is fine, but the tone needs to be down.

To clarify, we are generally not arguing for preferential transport of specific species. Rather, we are explaining the transport of non-subtropical gyre foraminifera into the South Atlantic gyre by transport, which is probably also transporting some gyre species away from our site, which is invisible to us. In addition, the subtropical gyre causes surface waters to converge at its centre, which will tend to import extra-gyre species into the gyre and our site.
Based on abundance maps from the ForCenS Database *G.bulloides* has highest abundances in the eastern South Atlantic (Fig. R1).

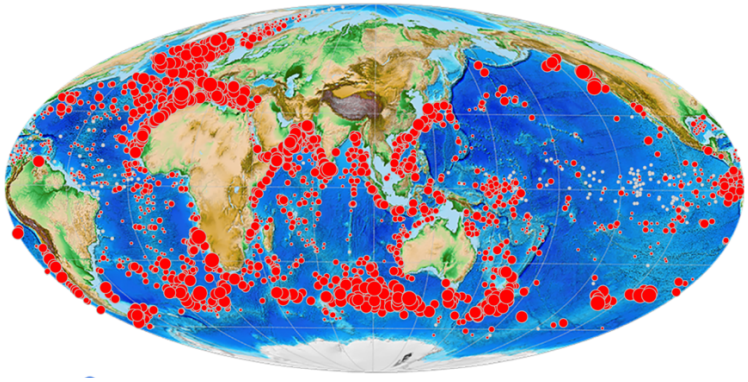

Fig. R1 – *G.bulloides* assemblage counts from Siccha and Kucera (2017), https://www.mikrotax.org/system/ranges-ForCenSbiogeog.php?search=Globigerina_bulloides&plotorder=ASC&scale=1&basemap=Gplatesbathymetry (accessed 18.11.24).

G.siphonifera has not a clear east-west distribution. However, it could be that it is their cooler-water genotype that is being brought in (e.g., Type IIa described by Darling and Wade (2008) see their Fig.9 and section 2.7). In contrast, *G.ruber albus* have highest abundances in the oligotrophic gyre in the southwestern Atlantic (Fig. R2).

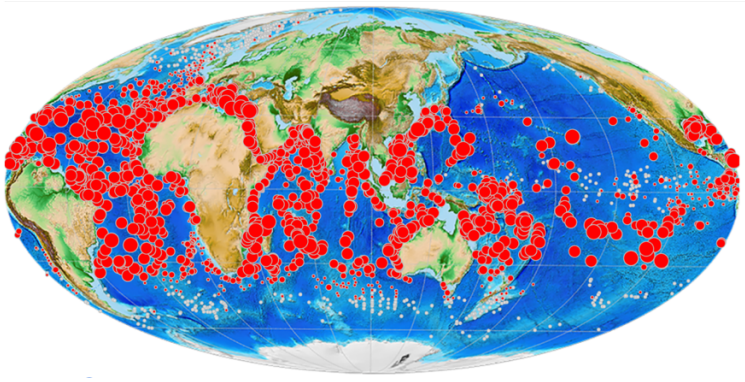

Fig. R2 – *G.ruber albus* assemblage counts from Siccha and Kucera (2017), https://www.mikrotax.org/system/ranges-ForCenSbiogeog.php?search=Globigerina_bulloides&plotorder=ASC&scale=1&basemap=Gplatesbathymetry (accessed 18.11.24).

If entire assemblages are being transported as the reviewer suggests, then the transported assemblages may be mixed with the local assemblages. However, while being transported and mixed with the water bodies, the ecological conditions will change and support some species and disadvantage others. The supported species (e.g., *G.bulloides* and *G.siphonifera*) may continue to grow and add to the local assemblage, while the others will decease and sink out of the water body, and not add to the local assemblage.

6. Geochemical proxy for photosymbiosis

I believe FB-$\delta^{15}N$ has great potential as a useful tool to distinguish fossil foraminiferal photosymbiosis. As with size-specific $\delta^{13}C$, not all tools are perfect, but their combination will enhance our understanding of the phenomenon. I would like to encourage the authors to emphasize the potential of this proxy. Specifically, the FB-$\delta^{15}N$ differs from $\delta^{13}C$-based reconstructions of photosymbiosis in that it can differentiate

symbiont species (dinoflagellate or not), which is a great advantage in reconstructing photosymbiotic partnership through evolutionary timescales. I understand that the manuscript addresses important aspects of the limitation of the proxy or points to be aware of, but the more positive argument for the usefulness of this proxy would make this paper more appealing.
Agreed, we added a sentence in the abstract and conclusion section to highlight this a bit more.

Technical corrections
Text overall: The style of in text references need to be checked. There are many parentheses within parentheses, and sometimes only one side parenthesis. Please check the journal format and correct them.
Checked and corrected.

L91: …by feeding on foraminiferal feeding on algal cells.
by foraminiferal feeding on algal cells
Corrected

L95: shell or test
Please keep consistent wording.
We use now tests throughout the text.

L97: …(Spero et al., 1991). Period is missing.
Corrected

L108: planktonic foraminifera
In this paper, the author uses “planktic” instead of “planktonic” in the title. Please keep it consistent.
Corrected

L156–165: In this paragraph, both chemical names and chemical formula are used. I suggest to use chemical formula consistently (nitrate in L156→NO3-, nitrite in L161→NO2-).
Done

L167: 250-400μm size fraction
Specimens for FB-$\delta^{15}N$ were picked from 250-425μm size fraction (L131). I suppose 400 may be 425, since the largest fraction is >400μm (L172).
We used 400μm as the upper limit for all isotopic analyses. We corrected the size fraction on Line 144 to 400μm.

L183: The title of this section is the same as the previous one. Probably something like “Age model”?
Oops, must have been a copy paste error… corrected it to “2.4 Age model”.

L217-218: This paragraph can be deleted since the discussion and related figure are all completed in supplementary materials.

We would like to indicate to the reader that seawater analyses results are not discussed in the main text and can be found in the supplements, thus we prefer to keep that sentence.

L246: 4.2...carbon isotopes in DSDP Site 516 ---> at DSDP Site 516 (for consistency to 4.1)
Changed all "in site..." to "at site..."

L294: foraminifer --> foraminifera (for consistency)
Corrected

L312: *G. menardi* --> *G. menardii*
Corrected

L325, 329: Please correct the spell of "dinoflagellate".
Corrected

L353: PON --> need to represent abbreviation (particulate organic matter) or unify the term to PN which is used in the text prior to this.
Changed it to PN

Caption of Fig. 2: *G. siphoniphera* (typo) ---> *G. siphonifera*
Corrected

Fig. 2d: The $\delta^{13}C$ of benthic stack cannot be seen clearly because of overlap. There appears to be no discussion on this profile, so it may be removed. In addition, the caption says "benthic stack of South Atlantic cores at shallow depths (Lisiecki et al., 2008)", but the one I found in the reference paper was for shallow North Atlantic sites. Is it correct?
Removed $\delta^{13}C$ (Lisiecki et al., 2008) from the figure and moved $\delta^{18}O$ benthic stack to the top. Adjusted figure caption accordingly.

Fig.4c: The labels of the vertical arrows should be reversed (higher $\delta^{13}C$ should be higher photosynthesis).
Done

Caption of Fig. 4: Distinction --> distinction
Corrected

Fig. 5: The category of *G. hirsuta* here is "chrysophyte or pelagophyte symbionts", but I think this should be symbiont-barren. Although Gastrich (1987) reported chrysophyte from this species, later on Hemleben et al. (1989) further analyzed this species and concluded that algae in this species should be prey. The other related paper to the authors study also categorize *G. hirsuta* to symbiont-barren (see Smart et al. 2018 for example). Likewise, please show the reference for *G. tumida* symbionts. I don't know whether this species has been investigated for symbiosis.

There does not appear to be a consensus in the literature yet on whether *G. hirsuta* is symbiont barren or has chrystophyte symbionts. Hence, we removed the vertical bar between symbiont-barren and non-dinoflagellate symbionts in Figure 5b.

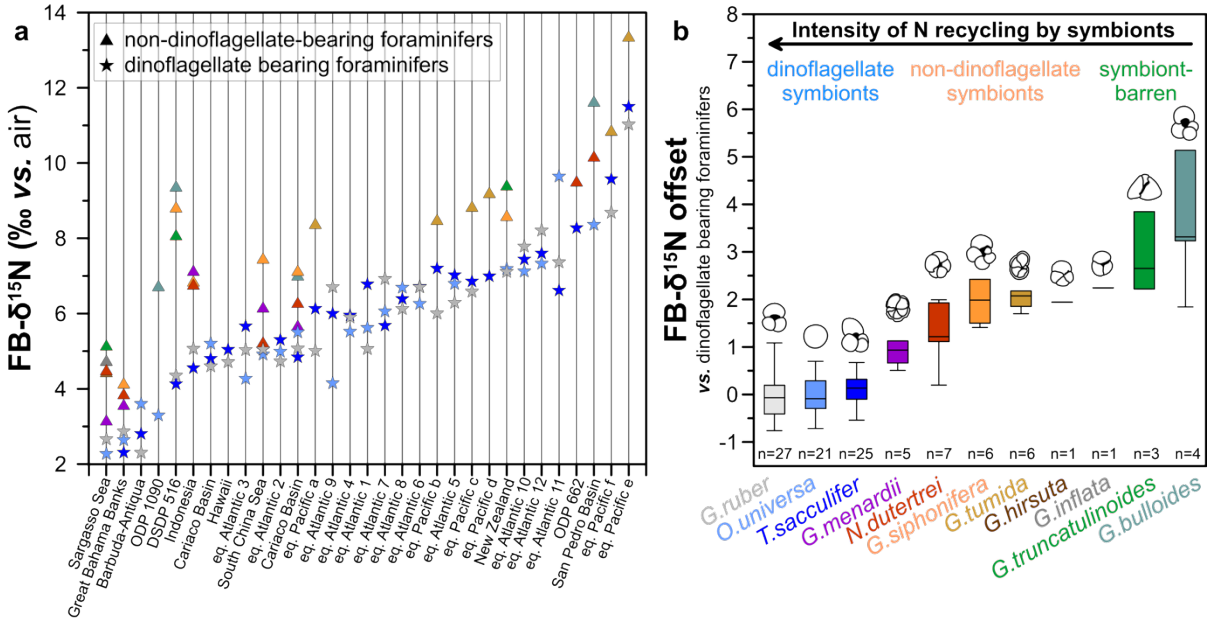

Fig. 5 updated (adjusted Figure 5b)

Done

Caption of Fig. 9: “dinoflagellate hosting foraminifera” ---> “dinoflagellate-bearing foraminifera” is better for consistency.
Done

Supplementary material 1st page, 1st section: ...compilation (Fig. S2 b,c,e) ---> Fig.S3
Corrected

Supplementary material 2nd page, 2nd row: *G. ruber* and *G. siphonifera* $\delta^{13}C$ seem to be higher than *T. sacculifer*... ---> lower (or more depleted)
Corrected

Caption of Fig. S2, S3: “(d) *G. bulloides*, ... and (f) *G. truncatulinoides*” ---> Opposite. (d) is *G. truncatulinoides* and (f) is *G. bulloides*.
Corrected

Table S1: *G.Siphonifera* (typo) --> *G. siphonifera*
Corrected

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