Response to the anonymous Reviewer 1:

Comment: "Review of *Friesenhagen* **Test-Size Evolution of the planktonic Foraminifera** *Globorotalia menardii* **in the Eastern Tropical Atlantic since the Late Miocene**, submitted to *Biogeosciences*. I believe this manuscript has the potential to contribute to the understanding of the test-size evolution of the *menardii* lineage. It provides a new, supported hypothesis to the previously studied giant menardii form observed in other locations, that can also be further tested. The data is produced using both reputable and novel methods, allowing for this hypothesis to be fully evaluated. The author is clearly well read on previous studies and results regarding the topic, and the manuscript is well written and concise. However, corrections need to be made to the manuscript; some sentences can be rewritten to make it easier to read and a final proof reading is needed. I have made some suggestions as follows:"

Response: I thank the anonymous reviewer for the comments and suggestions to improve the manuscript. A detailed answer to each of the suggestions is given below.

Comment: "L19 – remove comma after (ETAO)"
Response: This suggestion is adopted.

Comment: "L35 – remove "as" before "for example" and add comma after""
Response: This suggestion is adopted.

Comment: "L37-39 – "One interesting case in the long-term test-size evolution of PF was observed by Knappertsbusch (2007; 2016), when he investigated the test-size evolution of the G. menardii–G. limbata–G. multicamerata lineage since the late Miocene." – is hard to read. Suggestion: **"Knappertsbusch (2007; 2016) observed interesting long-term testsize evolution of PF in studies of the G. menardii–G. limbata–G. multicamerata lineage from the late Miocene."**

Response: The reviewer's suggestion will be used to improve the texts' flow.

Comment: "L48 – change watermasses to water masses (check throughout manuscript); separate to separates"
Response: This suggestion is adopted.

Comment: "L51 – The NHG"

Response: This suggestion is adopted.

Comment: "L58 – after the onset"

Response: This suggestion is adopted.

Comment: "L59 – ommitt comma after investigates"

Response: This suggestion is adopted.

Comment: "L63 – reword: it seeks new insight into the underlying… ; In this context, [add comma}"

Response: This suggestion is adopted.

Comment: "L64 – allow us to"

Response: Probably the reviewer refers to line 68. To follow to the used style of writing, I would like to avoid "us".

Comment: "L65 – should be: Here, the (change . to ,)"

Response: This suggestion is adopted.

Comment: "Figure 1.: check latitude numbers: looks like O not zero on some of the numbers"

Response: Indeed, the numbers are not coherent and will be corrected.

Comment: "L75: change "waters, the known habitat for"…"

Response: This suggestion is adopted.

Comment: "L77-78 – Reword: The core location is outside, or within the peripheral, of the NW African upwelling system (Fig. 1) and therefore, only marginally affected for the investigated time interval of the last 8 Ma (Weaver and Raymo, 1989). Thus, there is a relatively long-term water-column stability on the geological timescale at ODP 667."

Response: The suggestion is adopted.

Comment: "**Suggestion for section 2.2.**: omit table 1 – fig. 2 shows the same information and is easier to read. Write how many samples, and what resolution at beginning of section."

Response: I disagree with a removal of table 1. It contains crucial, basic information for scientists who want to reproduce this study. If the reviewer consists on this point or thinks that it would improve the readers flow, I would offer to move the table into the supplementary material.

Comment: "L109 & 111 – weighted should be weighed"
Response: This suggestion is adopted.

Comment: "L115 – P.A.S.I srl should be written in full for first use."
Response: "P.A.S.I." the abbreviation for the Italian company "Prodotti e Apparecchiature Scienze e Industria". It will be added to the manuscript.

Comment: "L130 – State developer for AMOR software"
Response: Information on the AMOR hardware system and software version 3.28 are given in Knappertsbusch et al. (2009), as was cited in L132. For clarification, I will add this citation in L130 as well. In addition, the program "AutoIt" (Mary, 2013. Dissertation) was used for an automated processing of the imaging. It will be added to the text.

Comment: "L163-165 – Sentence looks incomplete/editing error – double check bold section: "Knappertsbusch (2016) refers to the disappearance of *G. limbata* as a possible pseudo extinction because of the occurrence of singular specimens of menardiforms with seven chambers in the last whorl after **2.39 Ma. with seven chambers are accounted as *G. limbata*, a form which became extinct during the early Pleistocene.**""
Response: Indeed, a sentence was accidently split. It will be corrected.

Comment: "I think it would also be useful to mention the difference between *limbata* and *menardii* in the first paragraph, alongside the *multicamerata* differences."
Response: The correction mentioned above will solves this suggestion.

Comment: "L175 – change to "gridded files": obtained by plotting…"
Response: The reviewers suggestion will be used to improve the texts' flow.

Comment: "L208 – change to: "This is the simplest analysis for…""
Response: This suggestion is adopted.

Comment: "L210 – omit: "as will be demonstrated in the following section.""
Response: This suggestion is adopted.

Comment: "L224 – On average,"
Response: This suggestion is adopted.

Comment: "L285 – this is the first time *G. menardii cultrata* is mentioned and it is without context. Why is this important to note?"
Response: The plotted line should help the reader to compare CFDs. The use of the line suggested by Knappertsbusch (2007) to distinguish the more delicate *G. menardii cultrata* from the thicker keeled and obviously bulgier *G. menardii menardii* may also help to visually detect long-term shifts in the δX-δY morphospace.

Comment: "L284 – this information may be better in the figure caption"
Response: In my opinion, placing this information in the main text improves the likelihood to draw the reader's attention to this interactive figure. If the reviewer still recommends to move this sentence into the figure's caption, I will adopt his suggestion.

Comment: "L323 – change to: "the giant sinistral specimens ($\partial Y = {>}1000$ µm) appeared 0.5 Myr earlier, at 2.58 Ma, in the eastern tropical Pacific Ocean Site 503 than in the Atlantic Ocean""
Response: This suggestion is adopted.

Comment: "L326 – change striking to substantial"
Response: This suggestion is adopted.

Comment: "L344 – omit rather"
Response: This suggestion is adopted.

Comment: "L357 – possible correction: South Atlantic rings, they may have drifted closer to the coast of SW Africa."
Response: This suggestion is adopted.

Comment: "L367 – change to: which possibly resulted in unfavourable"

Response: This suggestion is adopted.

Comment: "L390 – within short time periods."

Response: This suggestion is adopted.

Comment: "L391 – omit classic case of fast"

Response: This suggestion is adopted.

Comment: "L394 – italicise plesio"

Response: This suggestion is adopted.

Comment: "I think is it safe to remove examples of other microfossils as it is irrelevant and you have enough examples with foraminifera"

Response: Mentioning this additional reference gives another example for a rapid evolution within 50 Kyr and shows that rapid evolution is also suggested for other planktonic organisms. If the reviewer still recommends the remove of this example, I will follow this suggestion.

Comment: "L403 – change only occur between 2.3 Ma …; omit "and not earlier or later?""

Response: This suggestion is adopted.

Comment: "L414 – correct reference format Author (date)"

Response: The reference format will be corrected.

Comment: "L816 – not sure if (ä) is meant to be there"

Response: This mistake will be corrected.

Response to the anonymous Reviewer 2

Response to "General comments"

Comment: "The manuscript 'Test-size evolution of the planktonic foraminifera *Globorotalia menardii* in the Eastern Tropical Atlantic since the Late Miocene' present a size record of *M. menardii* over the last 8 million years. Most notably, it shows an increase in size to a 'giant' *G. menardii* type in the last 2 million years. The manuscript explains this size change by investigating three possible hypotheses: influx of giant specimens from the Indian Ocean by Agulhas Leakage, a local evolutionary event or a response to oceanographic conditions. I have several major concerns regarding the methods and data interpretations."

Response: I thank the anonymous referee for the critical comment. Some of the reviewer's concerns may be caused by some unclear formulations in the script, others may be solved by clarifying that all the presented hypotheses are working hypotheses, which need to be further investigated in future studies. Detailed responses to the reviewer's critiques and suggestions are given below.

Comment: "The dataset is not suitable to test the first two hypotheses (Agulhas Leakage and Local Evolution), and evidence for the third hypothesis is inconclusive."

Response: The scope of this initial study is not to definitively prove or dismiss one of the three presented hypotheses, but to discuss the possibilities of these hypotheses as explanations, especially of the Agulhas Leakage hypothesis (ALH), and to identify directions for further investigations. In this context the very similar patterns of size evolution at 667A (eastern Atlantic) and 925B (western Atlantic), thousands of kilometres apart, are one argument in favour of the ALH. This pattern of similar size evolution within the tropical Atlantic as well as the Caribbean Sea was not known before.

Comment: "Additionally, the characteristics used for species identification (number of chambers in final whorl) are not sufficient to tell Menardiform species apart. There is a possibility that the size record presented here consists of multipl*e* Menardiform species and any changes in size could therefore be the result of a change in species composition, rather than a species-specific evolutionary event."

Response: This comment is most likely based on an unclear formulation in the manuscripts' Material and Methods chapter 2.4. As mentioned by the reviewer, there are several other menardiform species like *G. exilis* and *G. pertenuis* with 5 to 9 chambers in their last whorl.

These species are easily separatable from *G. menardii*, *G. limbata* and *G. multicamerata* and had been previously removed from the applied dataset for this study. The reason for the removal of *G. exilis* and *G. pertenuis* is that (1) their occurrence is too rare and sporadic to be investigated in this study and (2) their removal facilitates the investigation of the *G. menardii-G. limbata-G. multicamerata* branch.

These points will be discussed in detail in the section 'Response to "Methods"'.

Response to "Specific comments – Interpretation"

Comment: "The link between AMOC strength and size, which is presented as the best explanation for size increase at 2.0 Ma in *G. menardii*, is weak."

Response: The reviewer's comment revealed that some passages about the AMOC hypothesis may be misunderstandable. As written in the Conclusion, this hypothesis represents one possible explanation for the size evolution from 8 to at least 3 Ma due to the covariation of the AMOC strength (Dausmann et al., 2017 and as a new additional reference Karas et al., 2017) and the test-size evolution. However, during the Gelasian I postulate that the dispersal of a new *G. menardii* type via the Agulhas Leakage is the most likely cause for the observed substantial test-size increase. Text passages which may cause the unintended impression of the AMOC hypothesis being the favoured explanation for the test-size increase around 2 Ma, will be modified accordingly.

Comment: "The covariation between εNd and size is not significant at Site 925 and explains very little variation in the size record of Site 667 (Figure A8)."

Response: I am aware of the fact that the statistical significance of the covariation is low, but one cannot dismiss a certain similarity between εNd and the pattern of the maximum test-size evolution over larger time as is shown in Fig. 10 and Fig. 12 of the manuscript. The hypothesis was formulated as a working-hypothesis. A detailed discussion about the covariation is given further below.

Comment: "If Atlantic water column restructuring had a strong influence on size through accumulation of nutrients we would expect this effect to be visible everywhere in the Tropical Atlantic."

Response: In my opinion, the effect is visible in the tropical Atlantic, shown by the very similar pattern in size evolution of *G. menardii* in the western and eastern tropical Atlantic as well as in the Caribbean Sea (Fig. 12).

The proposed AMOC and thermocline hypotheses are an attempt to explain the observed test-size pattern within the Atlantic Ocean, but it is clear that further work and studies are needed to test these hypotheses. *Globorotalia menardii* is known to thrive within the thermocline and the chlorophyll maximum zone (e.g. Ravelo et al., 1990), so that a relation between the food availability and the test size can be proposed.

Comment: "Additionally, if size increased due to nutrient accumulation in the thermocline we would expect to see a size increase in all thermocline dwelling species at the same time. For as far as I know, no such increases are known for any other species."

Response: To my knowledge there is no quantitative study available that relates nutrient enrichment with size evolution in PF and none - besides these mentioned in the manuscript - which investigates the test-size evolution of any other PF in geological timescales in the tropical Atlantic Ocean which may give further evidence or reject the thermocline hypothesis. If the reviewer knows of any studies which could help to prove or disprove the hypothesis, I would be very interested and grateful, if the reviewer could share these.

Furthermore, if other thermocline PF species show another size evolution through time compared to *G. menardii*, this does not automatically mean that the thermocline hypothesis is wrong. Other thermocline species may well react in different manners to the respectively experienced ecological conditions. For example, they may have different diets, a different ontogenetic development and different lifetimes. Due to their ecological preferences, it is rather likely that they will react in different manners to AMOC-strength induced changes in the water column than *G. menardii*.

In addition, the AMOC is mostly likely not the exclusive environmental factor influencing the test size of *G. menardii*.

Comment: "Finally, if εNd and *G. menardii* size are linked we would also expect an increase in size in the interval 3.5-5.5 Ma, when εNd values were comparable to those of the interval with giant specimens. As *G. menardii* reached minimum size values in this interval, I am not convinced there was a strong link between εNd and size."

Response: I disagree with the reviewer. In the time interval from 3.5 to 5.5 Ma, a size increase at ca. 4 Ma is clearly observed, which parallels the εNd values. The impression of an opposite evolution of the AMOC strength and the test size between 4 Ma and 5 Ma is probably an artefact of the lower resolution of the εNd record by Dausmann et al. (2017) and the approach of linear interpolation of this record used in this manuscript. The stable isotopic record in Figure 3c of

Karas et al. (2017) suggest a relatively weak and stable AMOC from 4.5 to 5 Ma (see attached Fig. 1), precisely the time interval in which *G. menardii* shows a relatively small maximum size at Site 667 and 925. These results complement the data of Dausmann et al. (2017) and support the AMOC hypothesis. They will be integrated/added to Fig. 12 of the manuscript according to the attached figure 1.

A correlation between AMOC strength and the test-size evolution, even if not 1, is still recognizable. The system, however, is not strictly mechanistic and there are a multitude of subtle interrelationships between ecology and test size of *G. menardii*. A visual similarity between the εNd-trends and the test-size evolution cannot be denied.

In order to explain the missing strict, linear and cause-effect relationship, one may reason the following hypotheses:

1. It is assumed that the younger giant *G. menardii* type/form (0-2 Ma) may have occupied a (slightly) different ecology (ecological niche) in comparison to the ancestral Miocene/Pliocene form (2-8 Ma). It is a non-analogous situation. The younger type thus might have not been affected in the same way by changes of the AMOC strength than the older form.

Evidence for this explanation is given by a modified form of Fig. A8a (see attached Fig. 1). It shows the correlation between linearly interpolated εNd values and the maximum size from Hole 667A for the time interval from 0-2 Ma (blue points) and 2-8 Ma (orange points), which fall into two groups. Fig. A8a and A8b will be modified according to attached figure 2.

2. Due to the closure of the Central American Seaway, the Atlantic's hydrography and oceanography altered and the AMOC strength changed significantly (Haug and Tiedemann, 1998; Haug et al., 2001) which can explain the distribution of points in the attached figure 2.

Although the correlation seems weak, there is a visual correlation between εNd – trends and the test-size evolution from 3 and 8 Ma (Fig. 10, 12), which in my opinion is worth to be mentioned and to be tested in future studies.

Comment: "In the Agulhas Leakage hypothesis giant *G. menardii* are transported from the Indian Ocean, around South Africa into the tropical Atlantic Ocean. Although a giant form existed in the Pacific, no existing or new data is presented to suggest that giant forms also evolved in, or migrated to the Indian Ocean. A record of Indian Ocean *G. menardii* size is needed before the Agulhas Leakage hypothesis can be tested."

Response: I agree with the reviewer that data from the Indian Ocean are needed to check, if the ALH is possible. As mentioned in the manuscript at L385-386, a second study is currently in progress which investigates the test-size evolution in the Mozambique Channel.

At the moment, we have to rely on the data from Site 503 in the eastern tropical Pacific. The description of a second core would go beyond the scope of this paper. The present study is of preparatory nature, whose results are discussed in terms of the three hypotheses described in the manuscripts. It is a precondition for testing the ideas presented.

Passages in the text will be revised which may be misunderstood in a way that the data of this study can prove the ALH.

Comment: "The Local Evolution hypothesis discusses whether the giant *G. menardii* evolved locally through punctuated evolution. The resolution of the record presented here, with a sample resolution of 0.1-0.2 million years, is too low to test for sudden evolutionary events taking place in as little as 50,000 years (line 397). A much higher resolution record of the interval around (suspected) speciation is necessary to test for sudden punctuated evolution. A higher-resolution record could also help distinguish between the Agulhas Leakage and local evolution hypotheses: local evolution is likely a single interval with increasing maximum size, whereas leakage of eddies could have resulted in the sudden appearance of fully formed giant *G. menardii* several times. A higher resolution record with sample spacing of 5-10 kyr might be able to detect these differences."

Response: The reviewer is right: the data presented here cannot test this hypothesis, but please note that the dataset is not primarily intended to test a punctuated-evolution event. The hypothesis of a punctuated, evolutionary event was proposed by Knappertsbusch (2016) as an alternative hypothesis, which may explain the size increase in case the ALH fails (personal communication). The idea of this local evolution can only be validated by an additional, more detailed high-resolution record within the Gelasian stage, but is beyond the scope of the present work.

Please note that the cited literature examples for punctuated evolution in the paper rely exclusively on single cores and neglect the regional/geographical aspect of evolution. As far as I know, none of them tested their results in additional cores from different locations. Sudden changes in the morphology of a species may equally be caused by an immigration event from another location and so mimic rapid evolution.

Response to "Methods"

Comment: "The Methods describe species identification based on the number of chambers in the final whorl. However, chamber number alone is not enough to distinguish Menardiform species. The species descriptions in the Neogene planktonic foram atlas (Kennett & Srinivasan, 1983) state that *G. menardii* has 5-6 chambers in the final whorl, *G. limbata* has 6-8 and *G. multicamerata* has 8 or more. Additionally, *G. exilis* and *G. pertenuis* have 5-7 and 6-8 chambers in the final whorl, respectively. Therefore, specimens with 6 or fewer chambers, which the manuscript calls *G. menardii*, could be either *G. menardii*, *G. limbata*, *G. exilis* or *G. pertenuis*. The *G. menardii* size record presented in the manuscript could thus be a composite of several species, and any changes in size could reflect changes in relative species abundance rather than an evolutionary event."

Response: Please see the comments on page 1 and 2. Chapter 2.4 of the manuscript will be revised according as follows:

All menardiform specimens were identified on species level by illustrations in Kennett and Srinivasan (1985), Bolli et al. (1985) and in comparison with the reference collection to "49 Cenozoic planktonic foraminiferal zones and subzones prepared by Bolli in 1985 – 1987". This included also the identification of *G. exilis*, *G. pertenuis*, *G. miocenica*, *G. pseudomiocenica*, *G. tumida*, *G. merotumida*, *G. plesiotumida* and *G. ungulata*, which all are easily to distinguish from *G. menardii*, *G. limbata* and *G. multicamerata*. Diagnostic features included the size, the outer wall structure (porcelaneous appearance due to finer perforation), number of chambers in the last whorl and the δX and δY ratio. After species determination, forms like *G. exilis*, *G. pertenuis*, *G. miocenica*, *G. pseudomiocenica* and the *G. tumida* group were sorted out and not included in the present morphometric study. Thus, the dataset presented herein only contains specimens of *G. menardii*, *G. limbata* and *G. multicamerata*.

I am aware of the problem to distinguish *G. menardii*, *G. limbata* and *G. multicamerata*. The apparently most distinctive morphological character of *G. limbata*, its limbation of the chamber sutures on the spiral side (Kennett and Srinivasan, 1983), is difficult to recognise and is also observed in *G. menardii* and *G. multicamerata* (Knappertsbusch, personal communication; personal observation). In order to crystallise a possible cladogenetic pattern between *G. menardii* and *G. limbata*, the present study experimented with the pragmatic discrimination that *G. limbata* has only 7 chambers in its last whorl. In the absence of other taxonomically clearly distinguishable parameters this approach is to be understood as an experiment, which seems to work well. *Globorotalia limbata* became extinct at ca. 2.4 Ma (Wade et al., 2011), which falls together with the observation that the abundance of 7-chambered *G. menardii*-like

specimens drastically decreased and are only rarely found from 2.5 Ma to present (Knappertsbusch, 2007, 2016; this study). Another point favouring this definition is observed in Figure 5, 6 and 7 in the manuscript, which show that 7-chambered *G. menardii*-like specimens have an intermediate position between *G. menardii* and *G. multicamerata* and indicate a cladogenetic trend within the time interval from 5 to 4 Ma.

Comment: "*G. exilis* and *G. pertenuis*, which evolved from *G. limbata* are not mentioned in the manuscript, even though both were present in the tropical Atlantic at the time of the study interval (e.g. Chaisson & Pearson, 1997; Chaisson, 2003). These two species became extinct around 2.0 million years ago, around the time that *G. menardii* size increased."

Response: *Globorotalia exilis* and *G. pertenuis* were present but show exclusively episodical occurrences and were rare in number. Only the assemblage of the sample at 2.3 Ma was monospecifically composed of *G. exilis* and *G. pertenuis* specimens. For these reasons and to make morphological patterns more readable, these species were excluded from this study. The inclusion of these species would have gone beyond the scope of this paper and must be reserved to a separate high-resolution study.

Comment: "I wonder if this size change could in part be explained by a removal of smaller Menardiform species in the assemblage."

Response: I am not entirely sure if I understood the intention of this comment correctly. All intact specimens >63 µm of *G. menardii*, *G. limbata* and *G. multicamerata* were included in this study, as was described in section 2.3 and can be seen for example in figure 8 of the manuscript at 2.057 Ma.

In case the reviewer intended a different thread, I ask the reviewer to specify his comment.

Reference

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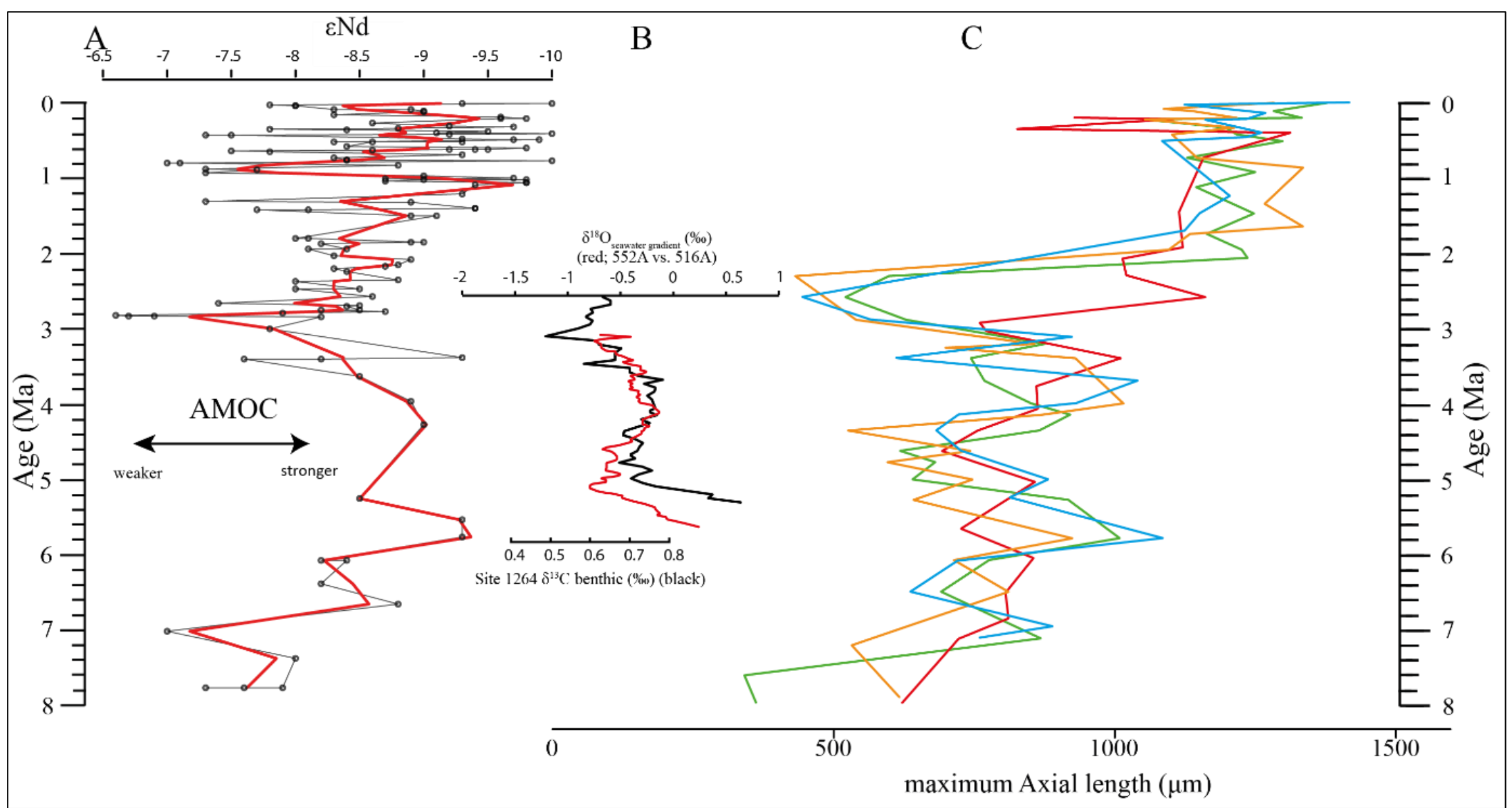
Figure 1: Comparison of seawater Neodymium isotope evolution (εNd), a proxy for the strength of the AMOC, and the maximum axial length of Globorotalia menardii. (A) Dausmann et al. (2017): εNd at Site 1088 in the southern Atlantic Ocean. The thin black line shows the original data, the red line a smoothed version, produced with the RStudios' command 'smooth.spline' at the value of 0.35. (B) Karas et al. (2017): the red line represents the $\delta^{18}O$ seawater gradient of the Sites 552A and 516A, while the black one is the benthic $\delta^{13}C$ curve from Site 1264. (C) εaximum axial length (δY) versus age (Ma). The green line represents the size evolution of Hole 667A (eastern tropical Atlantic; this study), orange of Hole 925B (western tropical Atlantic; Knappertsbusch, 2016), blue of Site 502 (Caribbean Sea) and red from Site 503 (eastern tropical Pacific) (Knappertsbusch, 2007).
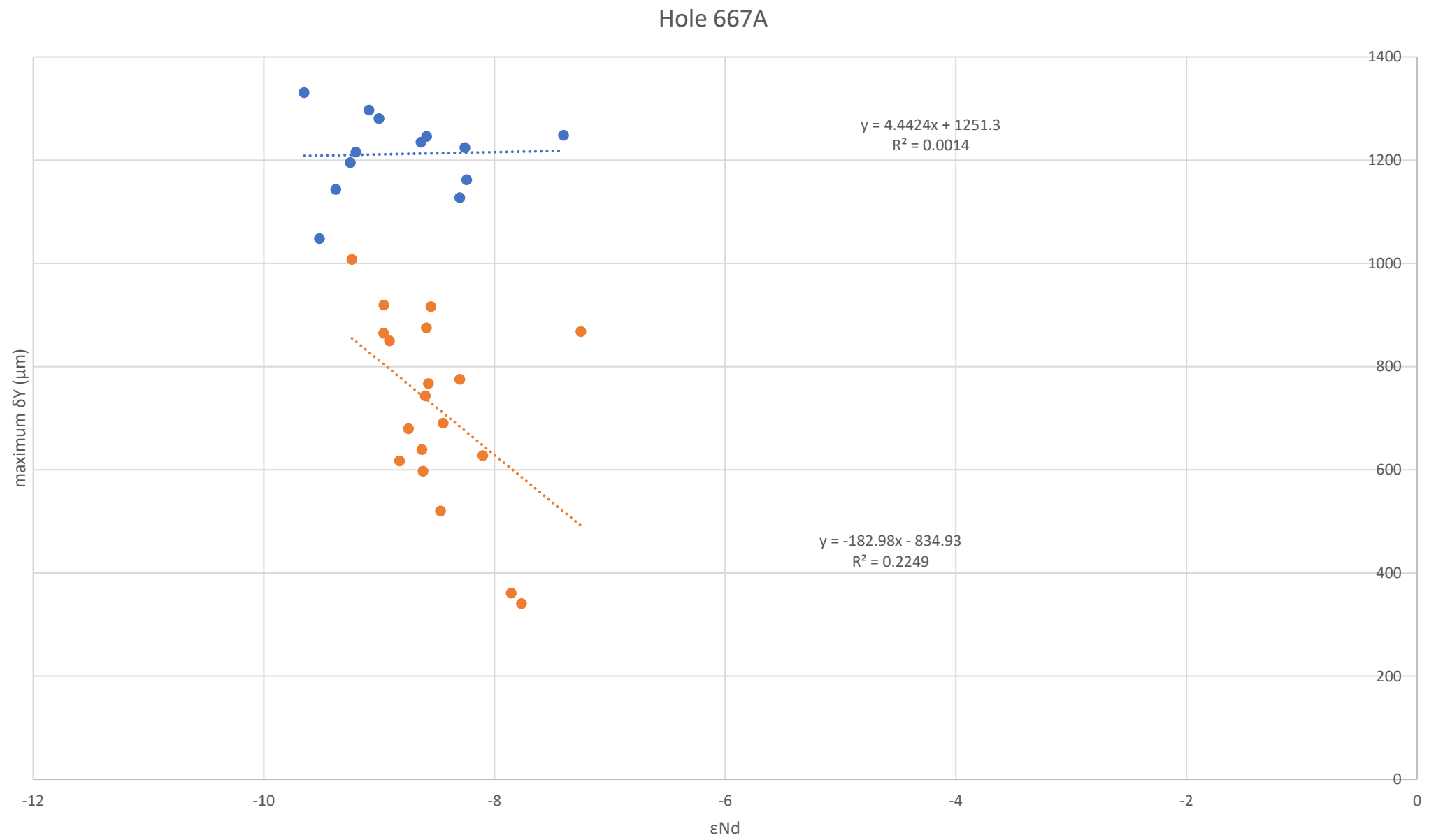
Figure 2: Plot of linearly interpolated εNd values versus the maximum axial length per sample from Hole 667A. Blue dots represent data from samples with an age from present to 2 Ma, while orange points indicate data for samples from the time interval from 2 to 8 Ma. This preliminary figure was generated with Microsoft Excel.

Response to the Comment by Nisan Sariaslan:

Comment: "In the title, instead of "the planktonic **Foraminifera** Globorotalia menardii", it should be "the planktonic **Foraminifer** Globorotalia menardii"."

Response: I thank Nisan Sariaslan for her comment. Following Lipps et al. (2011) "What should we call the Foraminifera?", the term "foraminifera" can be used either as a singular or a plural form. However, I will adopt her suggestion to follow one set of terms throughout the paper.