Reply on RC3
Daniel François et al.

Author comment on "Acidification impacts and acclimation potential of Caribbean benthic foraminifera assemblages in naturally discharging low-pH water" by Daniel François et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2022-91-AC3, 2022

We thank the reviewer for helpful comments and suggestions. Below, you will find our answers

Referee#3 Major comments:

Comment - The present manuscript by Francois et al., 2022 presents a field study of Caribbean tropical foraminifera in the Puerto Morelos reef Lagoon. Six stations were sampled in October 2011 along a broad natural pH gradient generated by submarine springs. The study of spatial variability on foraminifera fauna driven by pH gradient is an original approach. This work shows interesting results that corroborate knowledge already known/suggested in previous studies. However, this manuscript deserves to be restructured and clarified on some major points before publication.

It should be kept in mind that specimens come from the natural environment (not a controlled experiment) so multiple stresses can potentially influence calcification (salinity, eutrophication, pollution, warming...) these other parameters should be further discussed according to what is known about the site in previous studies.

Reply: To address this comment, we now performed multiple regression analysis considering all measured variables (carbonate system, temperature, and salinity). They were compared according to their contribution to the model’s Akaike Information Criterion (AIC), and the models with lowest AIC value (i.e., highest fit) were select to the analysis. Notably, consistent with previous studies we find the temperature and salinity have limited effects and the sites are not polluted so this is not a consideration.

We have added a discussion about salinity and for eutrophication/high nutrient concentration the following paragraph was already included in the manuscript:

“The springs from PM also have high nutrient concentrations compared to the open waters in the region (Null et al., 2014; Crook et al., 2016), however, near spring assemblages did not change towards opportunistic dominated assemblages, suggesting that the nutrient availability does not exert a major control at this site. Rather, the high-pH assemblages heavily dominated by small calcareous forms were replaced by larger symbiont-bearing species near the springs (Fig. 4a-e). Symbiont-bearing species are known to be sensitive...
to high nutrient loading, likely because of changes in turbidity/light regimes and their
dependence on algal symbionts to enhance growth and calcification (Hallock et al., 2003).
At PM despite higher nutrient levels the waters at the springs are clear and light regimes
are not reduced.”

Regarding warming, this is not considered to influence the species distribution as
temperature changed very little and for pollution we have analyzed the concentrations of
the metals (Paytan unpublished), and the concentrations were not significantly higher at
the discharge sites when compared to the control sites. We do not have a lot of heavy
metal data because it is a lot of work and when we found that the concentrations at the
springs were not high, we did not analyze anymore. Thus, we decided to not include this
information, but we could add a sentence along the lines – preliminary data for heavy
metal concentrations did not show significant differences between ojo and control sites
hence we do not attribute the changes in foraminifera calcification observed to impacts of
heavy metals.

Comment - There is a need for the bibliography to be more up to date especially those
published on LBF and µCT. I suggest this non-exhaustive list: Charrieau et al., (2022);
Kinoshita et al., (2021); Kuroyanagi et al., (2009); Fox et al., (2020); Iwasaki et al.,
(2019) ...

Reply: We thank the referee for the suggestions and the relevant bibliography was cited
in the manuscript.

Comment - In this paper, it is unclear about the use of live and dead fauna, if rose
bengal staining has been done you must describe in the manuscript the assemblage of the
foraminiferal fauna at each station and perform the ecological analyses on the live fauna.
The dead fauna cannot be treated with the live fauna. If you want to study dead fauna it
must be done in a separate section and clearly stated in the manuscript as "live fauna" or
"dead fauna". If you study dead fauna, you must describe the assembly of dead fauna.
The fact that there are few living foraminifera may be related to the seasonality of the site
(previous studies?). This site may be a place of sedimentary deposition, accumulation, and
currents... (1cm corresponds to what period? Previous studies?). You should discuss the
sampling method used if most of the living fauna live on substrates, it could be interesting
to think about a new sampling method?

Reply: The low number of fully stained foraminifera is probably not associated with
seasonality since it is a common pattern in the Caribbean and even in pristine (off-shore)
reef environments (Barbosa et al., 2009, 2012, Marine Micropaleontology) as most reef-
dwelling taxa live on phytal or hard substrates rather than directly on the sediments.

About the use of total assemblages (live + dead tests), this approach has extensively
been used in the literature in the last decades to assess mid-term responses of
foraminiferal assemblages to environmental conditions (e.g., water and sediment quality),
including for changes in carbonate system (e.g., Uthicke et al. 2013, Scientific reports,
DOI: 10.1038/srep01769). It happens that the accumulation of foraminifera tests in the
sediments integrate the effects of stressors over time (Hallock et al., 2003; Environmental
Monitoring and Assessment, DOI: 10.1023/A:1021337310386), and also of small seasonal
fluctuations of the assemblages, providing an ideal indicator of the foraminifera responses
by reflecting the prevailing marine conditions. (Scott and Medioli, 1980; Journal of
Paleoecology; DOI: http://www.jstor.org/stable/1304312).

It is important to note that the present research was also motivated by the results
presented in Martinez et al., 2018 (Biogeosciences, DOI: 10.5194/bg-15-6819-2018) that
also used total assemblages from Puerto Morelos to investigate the effects of low
pH/saturation state waters. To compare our results we followed the same approach.
About the study on phytal and rubble substrates it would rather address the species responses within a particular point in time, which wasn’t our objective. However, to expand our discussion we briefly discussed the stained counts, i.e., mainly dead, recently deposited tests (most had some degree of staining but were not fully brightly stained) because as stated above the species don’t live in the sediments. Specifically, we mentioned the behavior of the most abundant species, *Rosalina globularis*, comparing our results with Di Bella et al. (2022) that specifically observed a resilient behavior for this genus in the low pH venting sites of Panarea.

**Comment** - You need to clarify which data are common with the paper by Martinez et al., (2018). It seems to me that you have the same dataset or a selection of them. If you share other data from this previous paper, please indicate it clearly (this can also help to reduce the manuscript).

**Reply:** The carbonate chemistry, temperature, and salinity data from 7 samples were based on those presented in Martinez et al., 2018. Here the data set was complemented with 20 mid-ranges samples collected at the same day following the same protocols described by Martinez et al. (2018) but not reported there.

As suggested, this information was added to the manuscript.

**Comment** - Your data are related to the impacts of a natural pH gradient on a series of stations at a specific date (October 2011). You are therefore looking at spatial variability of foraminifera along a pH gradient and not at temporal variability. If you want to discuss temporal projections, I will discuss this in a discussion section. To discuss temporal projections, you need to be more nuanced because you need to know the seasonal variability of the living fauna and their interannual variability and species metabolisms (maybe you have some previous studies on this site).

**Reply:** We agree with the referee that the assemblages response are associated to a spatial variability of the environmental data, but as explained above the use of total assemblages also imply a temporal (i.e., generational) factor to the assemblages response. That is the data represents the response of multiple generations of forams accumulated probably over decades (representing the upper ~ 1cm of sediment).

**Comment** - The result and discussion should be restructured, and the discussion needs major parts or titles.

**Reply:** We have restructured the manuscript and sub divided the discussion section.

# Minor comments

**Comment** - The title does not indicate the content of the paper I would specify LBF or tropical and the study area (to be reconsidered in the light of the new orientation of the paper)

**Reply:** We have change the title to: “Acidification impacts and acclimation potential of Caribbean benthic foraminifera assemblages in naturally discharging low-pH water”

**Comment** - L41 CaCO3 = calcium carbonate

**Reply:** We changed accordantly.

**Comment** - Can you clarify what you call “small or smaller foraminifera”

**Reply:** it refers to the discussed functional groups of small miliolids and rotaliids, following
the definitions of Hallock et al. (2003) for sensitivity/stress-tolerance taxa and Murray (2006) for different test compositions.

**Comment** - L209 “live fauna” or “dead fauna”? clarify this section

**Reply:** We meant total assemblages (stained + dead). It was clarified in the manuscript.

**Comment** - L211 Table S1 corresponds to Raw data of functional and test type groups. I think there is a file problem where is the faunal description?

**Reply:** We thank the referee for nothing this. The faunal description was uploaded as an excel file, since it couldn’t be included in the word file due its size. It will be available for the final document.

**Comment** - L215 Considering a 3 % contribution cutoff. Why 3%?

**Reply:** For the SIMPER analysis a set of thresholds are tested and the final cutoff is defined considering the respective 2D plot with lowest stress.

**Comment** - L119 normally a minimum of 300 live foraminifera should be picked if the density is high

**Reply:** A minimum of 250 was defined following the methodology of Hallock et al. 2003, which was specifically designed for Caribbean reef ecosystems.

**Comments**

- L143 breakage and dissolution of the shells. Do you have a precise reference to do this work (quantitative approach) or is it a subjective approach?

- L154 Dissolution can affect live foraminifera and it has already been shown that some decalcified foraminifera can survive (ex. Charrieau et al 2018 Biogeosciences). To detect living foraminifera, it is either the coloration (rose bengal) or a mobility test to know if the specimen is alive or not.

**Reply:** We performed a quantitative approach using two general categories: Pristine and (2) dissolved tests, with the latter considering any degree of alteration (in most cases that was a small degree of dissolution).

**Comment** - L261 p-value = 0.00 not correct (p-value < 0.001 for example)

**Reply:** the expression was changed accordantly

**Comment** - L165 they are many papers about CTnumber please add references To compare µCT specimens, it is recommended to remove the ontogeny effect (growth-related), and therefore to compare the specimens they need to have the same size (standardized by the average of the maximum diameter of the individuals). It is always nice to see µCT on foraminifera, but you need to discuss that few individuals have been scanned and therefore be critical with the inter-individual variability.

**Reply:** The analyzed specimens presented a similar size and volume, so little or no ontogenetic effect was expected to influence the analysis and the inter-individual variability was low.

**Comment** - L248 For the CCA, one of the axes is not significant, it would be interesting to make an Ordistep preselection to select only the parameters which contribute to the CCA,
and to have the two significant axes. It would be necessary to revise the design of the figure to put the variables in another color for more clarity and to put the complete legend of all the parameters used. The legends of all figures should be complete.

**Reply:** We thank the referee for this helpful suggestion. The second axis is probably not significant because it is related to temperature and salinity, which were the parameters that did not influence significantly the distribution of the species (BIO env/global BEST and linear regressions).

Interestingly, the Ordistep preselection indicated both variables (temperature and salinity) as still significant, and in that case, when removed from the analysis, none of the axis were significant. It probably occurred as the remaining parameters (i.e., related to the carbonate system) have high collinearity, which should be avoided for this analysis.

Since it would no longer be appropriate, we chose to remove the CCA analysis of the manuscript.

**Comments**

- we need to be able to understand a figure without reading the text all the time.

-L63 Fig.4e and not 4a -L284 add fig. 5 in the first sentence

**Reply:** We thank the referee for noticing these mistakes, we changed accordantly and revised the figures legends.