

Dear Mr Sparagon,

Your manuscript entitled "Coral thermal stress and bleaching enrich and restructure reef microbial communities via altered organic matter exudation" has now been seen by 3 referees. You will see from their comments below that while they find your work of considerable interest, some important points are raised. We are interested in the possibility of publishing your study in Communications Biology, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

We therefore invite you to revise and resubmit your manuscript, taking into account the points raised. In particular,

- 1) you should add details and discuss the differences between the three different coral species.
- 2) A functional prediction of the microbiome might be difficult with the limited sequence data available, but could be included and very carefully discussed as well.
- 3) In general, some more details should be added to the methods, results and discussion as requested by the reviewers as seen below.

Please highlight all changes in the manuscript text file.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if you wish to discuss the revision in more detail or if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

At the same time, we ask that you ensure your manuscript complies with our editorial policies. Please see [our revision file checklist](#) for guidance on formatting the manuscript and complying with our policies. You will also find guidelines for replying to the referees' comments. You may also wish to review our formatting guidelines for final submissions [here](#).

Please use the following link to submit your revised manuscript, point-by-point response to the referees' comments (which should be in a separate document to the cover letter) and any additional files:

<https://mts-commsbio.nature.com/cgi-bin/main.plex?el=A2Cx7GVw1A4BQst5I7A9fdjkS2WRKVqpdktbOmRHvKAZ>

** This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage first **

When submitting the revised version of your manuscript, please pay close attention to our [Digital Image Integrity Guidelines](#).

We would expect revisions of this nature to take around three months, but appreciate that every situation is unique. We look forward to receiving your revised manuscript when it is ready, and will not enforce a hard deadline on this revision.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Best regards,

Tobias Goris, PhD
Associate Editor
Communications Biology
orcid.org/0000-0002-9977-5994

Referee expertise:

Referee #1: Coral ecology/diversity

Referee #2: Marine microbial ecology

Referee #3: coral microbiology

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The authors have presented a very interesting work on an understudied research area to answer the influence of DOM exudates from stressed corals on the community structure of the surrounding bacterioplankton. The authors observed the highest concentration of DOC exudate and greatest growth of bacterioplankton in the Heated treatment, and not in the bleached and heated treatment, as one would expect. This indicates that the onset of coral thermal stress is a very critical time, affecting the water column chemistry and the bacterioplankton community structure around the corals.

I have a few doubts and suggestions to make the MS clearer for readers.

Dear reviewer 1, we appreciate your time and comments. Thank you.

1) The authors may please clarify if there was significant difference in DOC exudates between the bleached+heated and the control group. Line no.275.

There were no significant differences in the DOC data, we have clarified that in the corresponding sentence on line 275 (now line 287).

2) Also, it will be better to mention the source of seawater (from wild or artificial sea water) used in the experiments in the first section of results.

We specify the source of the seawater in the supplementary information document, lines 34-35: "Influent water from a flow-through seawater system (sourced from a depth of 6 m directly adjacent to the Gump Station fringing reef) was pumped into the aquaria..." We added this information to the start of the results as suggested on lines 114-115, indicating the aquaria were filled:

"...with unfiltered water sourced from a depth of 6 m directly adjacent to the Gump Station fringing reef..."

3) The authors have used three different species of corals, but the results are not explained based on the specific species and no differences are highlighted. The authors have represented the inter-species differences only in term of symbiont density (Fig. 1D). What was the rationale to sample the coral species (*Pocillopora verrucosa*, *Acropora pulchra*, and *Porites rus*) and were there any differences amongst them in terms of DOM exudates and the resultant bacterioplankton community?

We appreciate the reviewers' insightful feedback on this issue and apologize for any ambiguities in our text. Rather than test for species-specific differences in DOM release/microbial growth in response to thermal stress/bleaching, we opted to combine the 3 corals species in individual aquaria to mimic the natural composition of coral

communities on Mo'orea (Edmunds, P of Moorea Coral Reef LTER. 2022. MCR LTER: Coral Reef: Long-term Population and Community Dynamics: Corals, ongoing since 2005. knb-lter-mcr.4.39 doi:doi:10.6073/pasta/913b1532594fcdf02de7671ed940b4c5) and the general coral community response to thermal stress/bleaching. We chose Pocillopora verrucosa, Acropora pulchra, and Porites rus due to their relatively high abundance on LTER1 adjacent to Gump Station. We mention this in lines 463-465 of the methods:

"To mimic reef-wide bleaching/thermal stress signals, two nubbins from each of the three coral species at a given bleaching phenotype were combined with unfiltered water in individual aquaria for a total of six coral fragments in each of the 12 aquaria", as well as in lines 32-34 of the supplementary information:

"To mimic reef-wide bleaching/thermal stress signals, two nubbins from each of the three coral species at a given bleaching phenotype were combined in individual aquaria for a total of six coral fragments in each of the 12 aquaria."

To clarify this point, at the beginning of the results, discussion, and methods we refer to coral exudates from these coral-community aquaria as "coral community exudates." We additionally clarify this on lines 115-118 at the beginning of the results section, stating:

"Rather than test for species-specific differences in DOM release and bacterioplankton response, we opted to combine the 3 coral species in individual aquaria to mimic the natural composition of coral communities on Mo'orea (Edmunds, 2022) and assess the general coral community response to thermal stress and bleaching."

We have also added a brief paragraph in our discussion to address the need for more research on coral species-specific responses to thermal stress/bleaching in terms of DOM exudation and bacterioplankton response on lines 374-385:

"In this study we used a mixture of three common Mo'orea coral species in each aquaria in order to investigate the coral community DOM exudation response to thermal stress/bleaching. Different coral species can exude different DOM quantities and compositions and yield slightly different microbial communities, although differences between coral species are smaller than differences between broader benthic "guilds" (Nelson et al., 2013; Wegley Kelly et al., 2022). The current setup does not allow us to investigate the species specific response to heating and bleaching. While we hypothesize that the response of increased and altered DOM exudation is a universal response on heating and bleaching by coral communities, future studies should investigate species specific differences. If there are substantial differences in DOM exudation and subsequent bacterioplankton growth between coral species, then the composition of the reef benthos might influence the reef-wide ecological impact and response on thermal and bleaching stress or its recovery."

Lastly, we used symbiont density at the time of collection (Figure 1D) to validate our visual assessment of bleaching status and justify the separation of corals into bleached or unbleached treatments. Different species have inherently different symbiont densities, thus the most accurate way to validate our assignment of bleaching status for corals was to calculate Symbiodinaceae densities on an individual coral basis and separate the different species.

Reviewer #2 (Remarks to the Author):

The paper of “Coral thermal stress and bleaching enrich 1 and restructure reef microbial communities via altered organic matter exudation” carried out a mesocosm heating experiment and bottle incubation compared how unbleached and bleached corals alter dissolved organic matter (DOM) exudation in response to thermal stress and subsequent effects on microbial growth and community structure in the water column. The results found that thermal stress of healthy corals tripled DOM flux relative to ambient corals and DOM exudates from stressed corals were compositionally distinct from healthy corals. These exudates significantly increased bacterioplankton densities and changed the microbial composition. This study confirmed that short-term thermal stress and long-term bleaching may extend into the water column, with altered coral DOM exudation driving microbial feedbacks that influence how coral reefs respond to and recover from mass bleaching events.

The manuscript was well organized and the data interpretation is convincing. Additionally, the figures and tables are presented clearly. This paper have good scientific values, for the coral protection. It can be recommended for publication after Appropriate modifications. Some specific comments as follows:

Dear reviewer 2, thank you for your time and comments.

1. The authors used three corals (*Pocillopora verrucosa*, *Acropora pulchra*, and *Porites rus*). Do different species have different responses, and are their responses universally applicable?

We appreciate the reviewers' insightful feedback on this issue and apologize for any ambiguities in our text. This was also brought up by Reviewer 1; we have pasted our response to them below.

Rather than test for species-specific differences in DOM release/microbial growth in response to thermal stress/bleaching, we opted to combine the 3 corals species in individual aquaria to mimic the natural composition of coral communities on Mo'orea (Edmunds, P of Moorea Coral Reef LTER. 2022. MCR LTER: Coral Reef: Long-term Population and Community Dynamics: Corals, ongoing since 2005. knb-lter-mcr.4.39 doi:doi:10.6073/pasta/913b1532594fcdf02de7671ed940b4c5) and the general coral community response to thermal stress/bleaching. We chose Pocillopora verrucosa, Acropora pulchra, and Porites rus due to their relatively high abundance on LTER1 adjacent to Gump Station. We mention this in lines 463-465 of the methods:

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2. In addition to the structure and diversity of microorganisms, their functions are the most important. In this experiment, the authors did not analyze the function of microorganisms. If

there were metagenomic data, this article would be more valuable. However, it is currently possible to supplement it with functional prediction. It is recommended that the author conduct a supplementary analysis of functional prediction.

We acknowledge that functional analysis of microbial communities is helpful in drawing ecological conclusions and thank the reviewer for emphasizing this point. However, we argue that functional prediction (for microbial communities) in this paper is both a) beyond the scope of this manuscript and b) has major drawbacks and is likely inaccurate for our 16S data, potentially yielding not only uninformative but misleading results. Specifically, because PICRUSt analysis, a common functional prediction tool for 16S amplicon sequencing data, estimates gene contents based on phylogeny, this analysis can be inaccurate for taxa without any representatives with sequenced genomes, as demonstrated for soils (Sun et al., 2020; Toole et al., 2021). Marine microbial communities, including those studied here in coral reefs, have especially low numbers of characterized bacterial genomes (Chen et al., 2021; Bodor et al., 2020), thus making PICRUSt inaccurate in these environments. Functional predictions will improve when more bacterial genomes from coral reef environments are made available, as evident from human data sets (Sun et al., 2020) and on ruminant data sets (using CowPI software; Wilkinson et al., 2018) thanks to rich data sets of fully sequenced genomes built from efforts such as the Human Microbiome Project (Huttenhower et al. 2012) and the Hungate 1000 collection (Seshadri et al. 2018), respectively.

Toole, D. R., Zhao, J., Martens-Habbena, W., & Strauss, S. L. (2021). Bacterial functional prediction tools detect but underestimate metabolic diversity compared to shotgun metagenomics in southwest Florida soils. Applied Soil Ecology, 168, 104129 <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-020-00815-y>

Sun, S., Jones, R. B., & Fodor, A. A. (2020). Inference-based accuracy of metagenome prediction tools varies across sample types and functional categories. Microbiome, 8(1), 1-9 . <https://www.sciencedirect.com/science/article/abs/pii/S0929139321002511>

Bodor, A., Bounedjoum, N., Vincze, G. E., Erdeiné Kís, Á., Laczi, K., Bende, G., Szilágyi, Á., Kovács, T., Perei, K., & Rákhely, G. (2020). Challenges of unculturable bacteria: Environmental perspectives. Reviews in Environmental Science and Bio/Technology, 19(1), 1–22. <https://doi.org/10.1007/s11157-020-09522-4>

Chen, J., Guo, Y., Jia, Y., Liu, G., Li, D., Xu, D., Wang, B., Zhou, L., Peng, L., Zhao, F., Zhu, Y., Sun, J., Ye, C., Wang, J., Zhang, H., Liu, S., Seim, I., Liu, X., Xu, X., ... Fan, G. (2021). Diversity, function and evolution of marine microbe genomes (p. 2021.10.26.465843). bioRxiv. <https://doi.org/10.1101/2021.10.26.465843>

3. Lines 312 to 320, the change of microbial species, such as enriched heterotrophic bacteria and putative pathogens, it can be explain using the r- or k- strategy in your discussion.

We have added an interpretation of our results through this lens in the discussion on lines 327-329:

“Through the lens of r- and K-selection, the release of surplus labile organic matter by stressed corals proliferates r- selected copiotrophs that rapidly outcompete the K-selected taxa that are often associated with oligotrophic marine, and specifically coral reef, systems.”

4. Lines 409-411, This sentence lacks evidence in the author's experiment and needs to be rewritten and referenced.

As part of the conclusion, these sentences were meant to reflect the hypothesis generated from this work and not current knowledge in the literature. We have rewritten lines 439-443 to better reflect this:

“Based on these results we hypothesize that at the coral colony level these effects may reduce a corals’ ability to resist and recover from thermally-induced bleaching. When our results are translated to a reef-wide scale, we predict that thermal anomalies and mass bleaching events could sharply alter reef water biogeochemistry, carbon flux, microbial communities, and ecosystem health.”

5. Section of line 501, how to detect the concentration and quality of DNA?

DNA concentration of the 16S amplicon library was assessed using a Bioanalyzer High Sensitivity DNA Assay (Agilent Technologies).

6. The network relationships of microorganisms play an important role in maintaining the health and homeostasis of coral hosts. It is recommended that the authors analyze the network co-occurrence patterns of different experimental groups.

While we agree that microorganisms play an important role within the coral holobiont, we would like to point out this is not the focus of our study as we did not analyze the microbiome of the coral holobiont, but rather the water column. While our sample size limits construction of separate networks for the different treatments, we have constructed a network of all OTUs and overlaid the various proportional abundances in each treatment on the node. This network can be found as supplemental figure 8 and is discussed on lines 240-246:

“Visualization of pairwise co-occurrence patterns of OTUs from the final time point reinforces these conclusions (Figure S8). OTUs enriched in the coral stress treatments including Alteromonadaceae, Pseudoalteromonadaceae, and Saprospiraceae clustered together in the upper portion of the network and showed a high degree of connectivity with each other (i.e. OTU 1224 and OTU 75), yet limited significant positive correlations

with OTUs in the network that were enriched in coral controls and/or negative water controls.”

We have also added a brief description of network generation to the methods sections on lines 604-605:

“The OTU co-occurrence network was generated using SPIEC-EASI (Kurtz et al., 2015) and visualized using Cytoscape (version 3.9.1) (Shannon et al., 2003).”

7. The entire experiment did not mention environmental parameters, and basic environmental parameters need to be provided in field experiments, including temperature, pH, dissolved oxygen, nutrients, etc.

We would like to emphasize that this experiment is first and foremost a lab-based aquaria experiment and bottle incubation; thus we did not collect a full suite of in situ environmental parameters. We used a HOBO data logger to collect high temporal resolution temperature and PAR data in the ambient water tables. Additionally, we collected point temperature measurements from the heated water table at 4 timepoints throughout the aquaria experiment.

All temperature and light intensity data are available in Supplementary Table 3.

Reviewer #3 (Remarks to the Author):

This is an interesting work. Through investigation of the impacts of DOM exudated from stressed corals (bleached, heated, and bleached + heated) on the abundances and community compositions of bacterioplankton in reef waters in mesocosm experiments, the authors discussed the negative impacts of DOM released by stressed corals on their own resistance and recovery. About the impacts of metabolomics components on microbiome authors analyzed the correlation on the basis of their distance matrices, any detailed compounds have been identified as major driver.

Dear reviewer 3, thank you for your comments and your time.

Minor comments:

Introduction:

1. L68-70, add citations.

We have added a reference for this statement.

Methods:

1. L466, I think you used a waterpick, not airbrush.

We did not use a waterpick, we used an airbrush. As reported by Szmant and Gassman (1990):

“Tissues were removed from the coral skeleton with a jet of high-pressure air and seawater from an artist's airbrush. This method generates a coral tissue/zooxanthellae slurry that is much more concentrated than that generated by the more commonly used Water-Pik method (Johannes and Wiebe 1970).”

Szmant, A. M., & Gassman, N. J. (1990). The effects of prolonged “bleaching” on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. Coral Reefs, 8(4), 217–224. <https://doi.org/10.1007/BF00265014>

2. L532, I would suggest using silva 138 rather than silva 132.

Our alignments use the software package mothur and their curated SILVA alignment databases. As noted within their current documentation, the v138 SILVA shows unusual artifacts and they recommend using the v132. As these two databases are only marginally different we have followed their advice.
https://mothur.org/wiki/silva_reference_files/

3. L537, “243 samples” is not correct.

The 16S amplicon sequencing library included samples from this experiment as well as other experiments that occurred at the same field site and time, totaling 243 samples. All samples were included in the sequencing library and thus the bioinformatic processing, including the low abundance OTU cull mentioned in line 537, was conducted on all 243 samples. We have clarified this on lines 543-545:

“A total of 243 samples from this experiment as well as other experiments that occurred at the same field site and time were included in this sequencing library.”

4. The methods employed in the analysis of the correlation between metabolomics and microbiome should be described.

We have added a brief description of our multivariate correlation analysis between metabolomic and microbiota datasets on lines 618-621:

“The correlation between metabolomic and microbiota data from this experiment was statistically tested using both Mantel Tests and Procrustes Tests in R (version 4.2.1) and visualized with a Procrustes plot.”

5. According to the legend of figure 1, there is acclimatization, while no information about the condition for acclimatization in the method section.

We mention the acclimatization period in the supplementary methods section, lines 25-27:

“After collection, corals were transported to the Gump Station research facility and acclimated to ambient conditions in a 1300 L flow-through water table for three days.”

We have additionally added this information to the methods section of the main text on lines 457-458:

“After collection, corals were transported to the Gump Station research facility and acclimated to ambient conditions in a water table for three days.”

Results:

1. According to your methods, I would suggest replacing OTUs with ASVs.

The term "ASV" is one type of Operational Taxonomic Unit, in this case defined as a unique sequence variant following denoising with DADA2, and therefore OTU is the more universally correct terminology, and makes clear that this is the taxonomic unit used in our analyses. In short, we are generating ASVs as the mechanism by which we define our OTUs and therefore opt to keep the term OTU in the manuscript.

2. Figure 1, no symbol “C”; I would suggest showing the densities of symbiotic algal cells for each coral species in figure 1D; change “symbiont cell” to “symbiotic algal cells”; the temperatures are not consistent with the content in lines 115-116 and 432-433.

The letter C is now shown in Figure 1. Temperatures are corrected and “symbiont cell concentration” is changed to “symbiotic algal cell concentration”.

Given that we combined multiple coral species in a given treatment to assess the general coral community response to thermal stress/bleaching, we think it is more logical to present the final Symbiodiniaceae densities as averaged across species in a given aquaria, as is currently represented in Figure 1D.

3. Figure 3A, is p value correct?

This p value is correct. We have added an additional clarification on lines 183-184, stating:

“...and overall DOM treatment had a significant effect on microbial community structure (PERMANOVA $F=4.637$, $R^2=0.72$, $p\leq 0.001$)”

Discussion:

1. This study has been performed in the small size water column in aquaria, I would suggest taking the hydrodynamic process and other consumers into account when discuss the impacts of exudates in the reef water.

We have added these points to the discussion on lines 401-405:

“This study used a sealed, controlled bottle system to accurately measure DOC and microbial growth characteristics. However, in situ conditions are vastly different from bottles or flow through mesocosms; physical dynamics like reef depth, water flow, and residence time, as well as the relative abundance of specific coral species on a reef, all likely impact the degree to which our observed findings translate to in situ impacts.”