nature portfolio

Peer Review File

Protein signatures predict coral resilience and survival to thermal bleaching events

Corresponding Author: Professor Brook Nunn

This file contains all editorial decision letters in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Decision Letter:

** Please ensure you delete the link to your author home page in this e-mail if you wish to forward it to your coauthors **

Dear Professor Nunn,

Your manuscript titled "Resilience in a time of stress: revealing the molecular underpinnings of coral survival following thermal bleaching events" has now been seen by 3 reviewers, and we include their comments at the end of this message. They find your work of interest, but some important points are raised. We are interested in the possibility of publishing your study in Communications Earth & Environment, but would like to consider your responses to these concerns and assess a revised manuscript before we make a final decision on publication.

Importantly, please consider these three major points when revising and indicate in your resubmission how you have addressed them specifically: 1) The methods and results sections need to be expanded on, all research should be reproducible, so more details around how temperature was manipulated would be ideal here. 2) Consider integrating the information in the supplementary materials with the main manuscript better. 3) Tone down the overall claims within the manuscript with respects to the biomarkers and their relation to resilience.

We therefore invite you to revise and resubmit your manuscript, along with a point-by-point response that takes into account the points raised. Please highlight all changes in the manuscript text file.

Please submit your point-by-point responses as a separate file, distinct from your cover letter where you can add responses to the Editors' comments that you do not want to be made available to the reviewers. Word files are preferred.

Important: The response to reviewers must not include any figures, tables or graphs. If you wish to respond to the reviewer reports with additional data in one of these formats, please add them to the main article or Supplementary Information, and refer to them in the rebuttal. Due to current technical limitations, any figures, tables, or graphs embedded in your rebuttal will not be included in the peer review file, if published.

We are committed to providing a fair and constructive peer-review process. Please don't hesitate to contact us if you wish to discuss the revision in more detail.

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 any cover letter), a tracked-changes version of the manuscript (as a PDF file) and the completed checklist:
Link Redacted
** 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 **

We hope to receive your revised paper within six weeks; please let us know if you aren't able to submit it within this time so that we can discuss how best to proceed. If we don't hear from you, and the revision process takes significantly longer, we may close your file. In this event, we will still be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Earth & Environment or published elsewhere in the meantime.

Please do not hesitate to contact us 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,

Christopher Cornwall, PhD
Editorial Board Member
Communications Earth & Environment
orcid.org/0000-0002-6154-4082

EDITORIAL POLICIES AND FORMATTING

We ask that you ensure your manuscript complies with our editorial policies. Please ensure that the following formatting requirements are met, and any checklist relevant to your research is completed and uploaded as a Related Manuscript file type with the revised article.

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An updated and completed version of our Reporting Summary must be uploaded with the revised manuscript
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Furthermore, please align your manuscript with our format requirements, which are summarized on the following checklist: Communications Earth & Environment formatting checklist

and also in our style and formatting guide Communications Earth & Environment formatting guide .

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In particular, the Data availability statement should include:
- Unique identifiers (such as DOIs and hyperlinks for datasets in public repositories)
- Accession codes where appropriate
- If applicable, a statement regarding data available with restrictions
- If a dataset has a Digital Object Identifier (DOI) as its unique identifier, we strongly encourage including this in the Reference list and citing the dataset in the Data Availability Statement.

DATA SOURCES: All new data associated with the paper should be placed in a persistent repository where they can be freely and enduringly accessed. We recommend submitting the data to discipline-specific, community-recognized repositories, where possible and a list of recommended repositories is provided at http://www.nature.com/sdata/policies/repositories.

If a community resource is unavailable, data can be submitted to generalist repositories such as figshare or Dryad Digital Repository. Please provide a unique identifier for the data (for example a DOI or a permanent URL) in the data availability statement, if possible. If the repository does not provide identifiers, we encourage authors to supply the search terms that will return the data. For data that have been obtained from publically available sources, please provide a URL and the specific data product name in the data availability statement. Data with a DOI should be further cited in the methods reference section.

Please refer to our data policies at http://www.nature.com/authors/policies/availability.html.

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

Summary: The authors provide a novel combination of proteomics and lipid quantification in thermally-challenged M. capitata that they have identified as resilient and susceptible to thermal bleaching, thereby demonstrating differences in proteins involved in immunity and metabolism that may contribute to, and possibly predict, the ability of corals to recover from thermal events. This project has high potential to impact and inform plenty of future research throughout the cnidarian field.

General Comments:
• The authors often use the term “microbiome” when apparently referring to only the bacterial community of the microbiome. This should be clarified throughout the text.
• The discussion needs to be condensed and streamlined significantly. As it is right now, it is a more detailed repeat of the results section. The authors should try to explain the bigger picture story and implications of their results, as well as the experiments needed to verify their results (metabolomics and pathogen susceptibility assays).
• Additional analyses (PICRUSt2 and METAGENassist) and data (depth of collection and outplanting to reef) should be added to the manuscript.

Specific Comments:
• L45: The sentence starting “Further, proteins identified…” is misleading. Firstly, “enriched” is a more accurate word than “amplified” when referring to proteins. Secondly, are you discussing differentially enriched proteins in this sentence? Did any proteins go the other direction?
• L68: Additional sources would be useful here, especially when the authors discuss reduced reproductive capacity.
• L77-78: The connection between host specificity of microbiomes and microbiome disruption leading to host mortality needs to be expanded. As it stands, that connection does not seem obvious to the reader.
• L80: or axenic? Though the authors should note that gnotobiotic models for cnidarians are currently being developed (see Costa et al 2021: https://doi.org/10.3389/fmicb.2021.637834)
• L92: pre-bleaching once the stressor is removed? At this point, it might also be useful to define the terms resilience, resistance, and tolerance as they will be used throughout the text.
• L98-100: These are some of many possible contributing factors (e.g., life history, sex, etc). It might be worthwhile to mention that the factors you list are some of many possible factors
• L100: I am missing a connection between the sentence that ends in line 100 and the sentence that starts in line 100. The introduction of “immune function” seems sudden and not self-explanatory. Removing the parenthetical portion of the second sentence will help, as it is unnecessary (explained in the third sentence of the paragraph).
• L104: Again, a connection is missing. Why is pre-bleaching molecular physiology key to determining resilience? It is not obvious here, as it could very well be something to do with the response to bleaching or the sym type hosted (e.g., what has been proposed with Durusdinium as low-photosynthate but high-stress resiliency, though this could also be debated).
• L138: “contributing to bleaching resilience” is directional, but the differences in protein enrichment may not be actually contributing, just correlating or in response to the thermal stress/bleaching event

• L164: I’m not sure whether using the word “community composition” is accurate for the analyses that you’re performing, as you’re not doing metabarcoding (e.g., ITS2) to obtain a more total community composition makeup
• L166: Is the symbiont density measured agnostic of symbiont type? As in, is it total symbiont density regardless of symbiont type? Because different sym types may be hosted at different densities which may affect host physiology/molecular physiology.
• L166-168: I wonder if the sym density decreases at the same rate for both sym types tested? Is there a way to measure this?
• L170: I wonder if the composition/domination changed throughout recovery
• L249-251: In Figure 2f or in this section, it needs to be made clear that the Bleached (B) corals are all from the heat stress T2 and the Non-Bleached (NB) corals are from the control T1.
• L285: Further analyses should be performed on the 16S data, for example PICRUSt2 and METAGENassist. These further analyses will help the authors investigate functional differences in the bacterial communities.

• L297-299: I wonder if there are some stats you can do on these differences/similarities in symbiont composition? Yes, both the resilient and susceptible populations had both D- and C-dominated corals, but were the compositions indicative of anything? What about any background symbionts present?
• L300: More proteins were detected, but what about peptide differences? The differences in proteins may be explained by a lack of difference in peptides (reflective of either transcription or differences in sequencing depth).
• L301: Is the statement “a common response to exogenous stressors” referencing translation?
• L310: I’m not sure if these differences “dictate” survival; it could be other things… life history? Reproductive capacity? Protein modifications? Also, the authors have not demonstrated that antiviral mechanisms are active (by pathogenic challenge), but have only demonstrated that some proteins/pathways are differentially regulated. In the coral immunity field, it is very common to conflate what has been qualified as molecular “immunity” pathways in higher order taxa with molecular immunity in more evolutionarily ancient metazoans. The authors should take care to make this clear in the text.
• L319: This makes it sound like the susceptible corals aren’t performing heterotrophy… I think you’d have to do some behavioral assays and/or isotope tracking or something to make these claims
• L346: Is there a citation for more diverse microbiomes supporting coral immune responses? Are you talking about

molecular immunity or pathogen response/susceptibility?
• L402-403: The phrasing of this sentence makes it sound like you continued to measure Urease after T2. Did you? Does it increase at T2 compared to T1 in resilient corals?
• L409: other types of microbiome analysis would help this claim (picrust2/metagenassist)
• L433: The authors should be careful throughout the section around line 433 to not make claims that they have not demonstrated. Differences in pathogen immunity have not been directly demonstrated in the present study, and mucins may be involved in functions beyond pathogen-immunity.
• L473: Did the authors observe that resilient corals activated heterotrophy and the susceptible ones didn't?
• L506: This sentence needs to be changed. The authors don't know whether T2R did this because they don't have pathogenic data; the immune system is complicated and these proteins may be doing something completely different
• L549: It would make more sense to the storyline of the discussion if there was an "immune" section
• L565: be more specific with what you mean with "protein" and "microbiome" here
• L569-on: I don't think you can say for sure that these are biomarkers of resilience with only n=12
• L577: how would these things be developed?
• L583: has the rice grain size thing been verified?

• L602: depths of collection?
• L606-610: Forgive me if I am missing something, but how does 2 deg every day for four days only take you from 28 to 30?
• Are there error bars or something to add to the down-slope line in Fig1b that could demonstrate differences in bleaching rate?
• L618: depth?
• L643-645 ish: site something for these sym types? Its2 seq?
• L687-689: The development of the p-values needs to be clarified. Did the authors assign these p values themselves or are they from statistics?
• L725: Picrust and metageneassist

Reviewer #2 (Remarks to the Author):

The manuscript presented by Nun and collaborators represents a complete insight into understanding why the differences in the recovery capacity of different coral colonies of the same species are distributed in a particular site. This is of special interest since it is assumed that at the species level, corals obtain the same tolerance to stressors; however, at the time of a bleaching event, the same species may have different responses. The manuscript is very well written, and the information is pertinent and flows within the text. Within the manuscript, the goal, the methods used (both experimental and laboratory), the results, and the discussion that not only covers the results but also seeks a relationship between the markers that were used are clear. Therefore, my comments are minima, and the manuscript can be considered for publication after a minor review.
Line 61. the thermal threshold is not only for a given coral species, but also for the given regional and local conditions.
Line 104. The bleaching response is the visual evidence of the stress, regardless of the physiological response; therefore, it would not be more accurate the therm tolerance threshold or resistance threshold.
Line 141. Is the term rejection accurately used (in general along the manuscript)? the corals already have "accepted" the symbiont, so they are trying to discard the already accepted symbionts.
Line 151. Clarify the ambient temperature (even if it is included in the materials and methods section.
Line 152. Does the 30°C have no fluctuation at all?? why all the temperature data does not include standard error or standard deviation??
Line 289. thermal-induced or trigger bleaching response?
Line 344. because it is considered that the process of energy is being stored; It has been shown that organisms that are subject to stressful conditions and even more so that have the ability to cope, are characterized by a higher metabolic rate (See Seibel and Drazen 2007), so that energy can be used as part of the energy I would say, and not as a reservoir.
Line 345. If the antiviral response is triggered by the thermal stress, the corals should be less susceptible to diseases; however on the contrary it has been recorded that during the bleaching response corals are more susceptible to be infected (e.g. Croquer 2009, Weill et al. 2009, Brand and MCManus 2009; Miller et al 2009). So, it has not been explored whether these enzymes may have other functionalities as metabolic repairers or any homeostasis balance pathway rather than anti-virals?.
Line 487. Review the citation format.
Lines 559-574. It is interesting that these markers want to be applied for management and conservation; However, it would be important to describe the limitations, mainly in cost, that this would have, what would be a possible proposal.
Line 606. Is the temperature constant and without any variation?
Line 609. Again, Since the research is based on the response to temperature regimes, the variation that could exist both during the acclimatization and stress periods must be described.

Reviewer #3 (Remarks to the Author):

Summary
The manuscript "Resilience in a time of stress: revealing the molecular underpinnings of coral survival following bleaching

events" by B Nunn and colleagues investigates the mechanisms underlying resilience and susceptibility of the coral Montipora capitata following an experimental bleaching event. The authors generated mass-spectrometry based proteomic data, along with characterizations of coral microbiomes, total lipids, symbiont density, and symbiont genera. The authors identify microbiome diversity differences in resilient and susceptible corals prior to bleaching, and identify differentially abundant proteins relevant to the holobiont stress response. Additionally, the authors link protein abundance prior to bleaching with abundance after bleaching, with potential importance for predicting coral resilience ahead of bleaching.

This manuscript represents a significant research effort and contains interesting data (particularly integrating proteomics with other more often measured phenotypes) that are relevant to the field of coral biology. However, I do have several points of concern that I feel need to be addressed before this manuscript can be published. Primarily, I feel that the manuscript does not contain sufficient detail in the methods and results to fully interpret or facilitate reproduction of the data presented. I highlight specific lines in my detailed comments below, but in general the results are missing important statistical analyses and the methods need more detail throughout. Specifically, for the microbiome analysis, I found what seem to be discrepancies between the supplemental information and the main manuscript, and ultimately feel that all of the details presented in the supplement are necessary to include in the main manuscript. Additionally, the authors make broad claims in the abstract and introduction about presenting a "multi-factor approach to identify whole-organism biomarkers of resilience". However, from my perspective the only convincing biomarkers you present are the protein data, as creating biomarkers from overall differences in microbiome diversity would be challenging (see specific comment below on the discussion). Additionally, the authors highlight the importance of microbiome diversity throughout the manuscript, and cite Figure 4a as illustrating such differences in microbiome diversity at the end of the discussion, but from my understanding of the legend and the data included Figure 4a does not seem to include any diversity metric but rather transformed counts of specific taxa. Faith's PD is presented in the results but not in any figure that I found, which in my view would be the more appropriate visual representation of these important microbiome diversity differences. I suggest being more specific in the claims about identifying protein biomarkers of resilience throughout the manuscript or adding in a more thorough presentation of the differences in microbiome diversity.

For these main reasons, along with my detailed comments below, I recommend that this manuscript be accepted following major revisions. In addition to my main points highlighted above, I have included line-by-line suggestions below that should be addressed before publication.

Abstract
L37: "16S rRNA of the microbiome" seems an incomplete way to describe this method, I suggest "sequencing of 16S rRNA to characterize coral microbiomes" or something similar.

L39: Specify 'protein biomarkers' here

Introduction
L68: I suggest adding a citation here for the statement about widespread mortality.

L89-92: Please add citations for both of these sentences here.

L109: Please add a citation for this statement.

L135: It is unclear what "semi-quantitative proteomics" means here, I suggest defining it either here or in the methods to highlight how it differs from a fully quantitative proteomic method.

General Comment: You mention that you outline a multi-factor approach to identify whole-organism biomarkers of resilience, but after reading through the manuscript in its entirety I do not agree that you accomplish this. You identify protein biomarkers that are potentially related to resilience, but no other factors are included in my opinion. I suggest re-phrasing to be more specific about protein biomarkers.

Results
L150: Were these 74 Montipora capitata confirmed to be genetically unique individuals? Did you check for clones in your dataset? Same comment for the 6 resilient and 6 susceptible colonies that you select – were you able to confirm that they were unique genotypes?

L150-153: It is unclear here whether you included a genotype control in this experiment, but from looking at the methods it does seem like that was the case. I think that is worth specifically mentioning here.

L158: Please define 'susceptible' as 'S' here, similar to what you do on line 159 for resilient corals.

L166-172: You are missing p-values for this entire paragraph, please add in results of a statistical analysis to compare the symbiont density values across treatments and time.

L167-169: Please provide the rate of decrease here and a statistical test to show that there was no difference in the rate of decrease between the groups.

L200-201: Here you mention that you only report p-values for significant results, but I do not see any p-values in this paragraph. Please add them. Also, I do not think this is an appropriate approach for the other physiology metrics that you present (see earlier comment L166-172), and all p-values should be included in the results.

L221-222: I'm not sure if I am missing it, but I'm unclear where you previously mention a protein involved in symbiont degradation. Please clarify.

L227-240: Please include a couple of extra references to Figure 3, those are missing from the second part of this paragraph.

L248-249: Again, missing p-values for this statement, please add them.

L251: Be more explicit about the two variables here, I am pretty sure you mean the interaction between bleaching status and time point but will be helpful to clarify for the reader.

L255-266: Are there figures to cite for these comparisons of Faith's PD? I'm unclear of where to find these data, which doesn't seem appropriate since you make large claims about the importance of microbiome diversity and its role in coral resilience. You cite Figure 4a in the final paragraph of the discussion to highlight differences in microbiome diversity, but this seems to just be abundance (i.e., counts) of a few taxa, and not representative of the metrics of diversity that you highlight in the results. I think that presenting these data in a figure would be most appropriate with the attention that you pay to the importance of microbiome diversity.

Discussion

L296-299: Instead of making this qualitative statement, I think you should include a statistical test to specify this point. For example, you could run a linear model relating the proportion of Durusdinum hosted by an individual pre-bleaching to mortality post-bleaching

L317-318: But in the results you state that this difference is not significant prior to bleaching, so this statement feels quite misleading. You could include the actual mean values of lipid content between groups in the results, and then clarify that the differences were 'trending' or something similar to this point in the discussion here. Regardless, the p-value and additional information needs to be included for the reader to interpret these results.

L411: Change 'advent' to 'onset'.

L485: I don't think the word 'yolk' is appropriate to describe coral gametes, I suggest removing the word entirely and the sentence will make more sense.

L487-488: Formatting of your references in this sentence is not aligned with the rest of the manuscript.

L558: In this coral management and restoration implications section, you elaborate in great detail on how a biomarker assay would work for proteins, but don't mention details of the microbiome biomarkers at all. I am not convinced that quantifying diversity of the microbiome would serve as a biomarker, and instead you would need to have directly linked particular microbial taxa to bleaching tolerance. Please remove the microbiome biomarker discussion here, and focus instead on the proteins as you do have more clear evidence for those proteins.

L586: change 'analyses' to 'sequencing'

Methods

L602: Please include the depth range that corals were collected from, as the context of depth and therefore light environment is important to contextualize thermal tolerance.

L602-626: Please indicate the light conditions that these corals were kept under for acclimation and for the experiment. Did anything change between acclimation and experiment? How do these light levels compare to what the corals experience on the reef?

L602-626: While you have included the target temperatures for the treatments, I don't think it is appropriate to point the reader to the supplemental data file to look for the actual temperature. Please either include a supplemental figure that plots temperature over time or summarize the actual treatment temperatures in this paragraph.

L611-612: Please include how many replicate tanks you had per treatment.

L619: What depth were these tables placed at, and does it match the depth range the corals were collected from? Please add more specific details here.

L640: Please add the detail of what wavelengths you measured absorbance at.

L640: "triplicate ground coral tissue" is confusing here – do you mean you conducted triplicate counts from one slurry of ground coral tissue? Or did you grind three different portions of the coral sample and conduct individual counts from those?

Please clarify.

L653-657: Please add information about the proportion that defined a community to be Durusdinium-dominated or Cladocopium-dominated, was it just >50%? And similarly, what defined a “mixed-community”? Also, we have a lot of evidence that particular species of Symbiodiniaceae really matter, are you able to distinguish the actual species of symbiont here or is this combining multiple species of each genera? If these characterizations include multiple species within each genera, I suggest changing the wording to be Durusdinium/Cladocopium spp.

L659-663: Please include details on the statistical analyses that you used to look for significant differences in lipids across your groups. It is unclear to me where to find this information.

L682-683: Please indicate where you obtained this reference transcriptome (i.e., GenBank accession information).

L701-725: You are missing a lot of details necessary to understand your microbiome analyses, and some of the details are not consistent between the main text and the supplement. Overall, my suggestion is to replace what you have in the main text with your supplement, as that contains all of the information I am looking for. But, here are a few examples of some issues I have with this section:
• You mention Mr. DNA for library prep and sequencing in the main text, but Molecular Research LP in the supplement. Please confirm what was actually done for sequencing.
• L713: You are not technically reporting “read depth” here, just the average number of reads per sample. Please add in the average read depth in addition to the total number of reads.
• L714: QIIME 2 and DADA2 are separate software, but QIIME 2 integrates DADA2 through a plugin (see info here: https://docs.qiime2.org/jupyterbooks/cancer-microbiome-intervention-tutorial/020-tutorial-upstream/040-denoising.html). Please update this sentence to be more clear.
• I strongly recommend including details on rarefication in the main text, as you only have it in the supplement.

Figures
Figure 1:
Please clarify why the sample size changes from n=6 to n=4 for symbiont community composition of susceptible corals. In general throughout the manuscript, I got confused with genet- and ramet-level replication. Please add clarification of the sample size of individual genotypes and the number of fragments per genotype throughout the manuscript as appropriate.

Figure 2:
L788: (‘grey boxes “B” boxes’) is unclear as written. I’d recommend changing ‘boxes’ to ‘bars’ in this legend since it is a barplot. Also, I think you could just say something like ‘grey bars with “B” labels’ and that would be more clear.

Figure 3:
L792: Please define NSAF here.

Figure 4:
Please add a label to the legend that indicates the significance of the size of each dot. It seems to me like you could just say “log-transformed counts” and then include the same details you have about those counts being phylum-level in the legend.

Figure 5:
Similar to Figure 3, please identify NSAF here.

Version 1:

Decision Letter:

** Please ensure you delete the link to your author home page in this e-mail if you wish to forward it to your coauthors **

Dear Professor Nunn,

Your manuscript titled "Resilience in a time of stress: revealing the molecular underpinnings of coral survival following thermal bleaching events" has now been seen by our reviewers, whose comments appear below. In light of their advice we are delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment.

We therefore invite you to revise your paper one last time to address the remaining concerns of our reviewers. At the same time we ask that you edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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Please review our specific editorial comments and requests regarding your manuscript in the attached "Editorial Requests Table".

*****Please take care to match our formatting and policy requirements. We will check revised manuscript and return manuscripts that do not comply. Such requests will lead to delays. *****

Please outline your response to each request in the right hand column. Please upload the completed table with your manuscript files as a Related Manuscript file.

If you have any questions or concerns about any of our requests, please do not hesitate to contact me.

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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

Alice Drinkwater, PhD
Associate Editor
Communications Earth & Environment
@CommsEarth

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors of the manuscript "Resilience in a time of stress: revealing the molecular underpinnings of coral survival following thermal bleaching events," have carefully and thoroughly responded to all reviewer comments and made

significant improvements to the manuscript as a whole. The changes have introduced more nuanced and impactful analyses of the data. This work continues to represent a novel and insightful analysis for the field. Based on the changes and explanations provided by the authors, I recommend that this manuscript be accepted for publication.

Reviewer #3 (Remarks to the Author):

I would like to thank the authors for their time and effort in thoroughly addressing my initial concerns in the revised version of their manuscript. I am happy to accept the revised manuscript without any further revisions.

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Dear Reviewers,

We would like to thank the reviewers for their detailed and constructive feedback on our manuscript, “Resilience in a Time of Stress: Revealing the Molecular Underpinnings of Coral Survival Following Bleaching Events.” The insights provided by the reviewers have significantly strengthened our work, enhancing both the clarity of our findings and the depth of our interpretations. We have carefully addressed each of the comments below **in BLUE** and incorporated substantial revisions throughout the manuscript, including the addition of new analyses, figures, and more precise language to clarify our results and their broader implications for coral resilience and conservation.

We would also like to offer our sincere apologies for the tardiness of our response. Due to the additional analyses requested by the reviewers and the fact that all currently contributing authors are principal investigators with active teaching responsibilities, we encountered significant time constraints. Unfortunately, no students or postdoctoral researchers were available to assist with this work, resulting in delays. We appreciate the reviewers’ patience and understanding as we worked to complete these comprehensive revisions.

This manuscript presents an integrated systems-biology approach to investigate the physiological and molecular mechanisms underpinning bleaching resilience in the coral *Montipora capitata*. Through quantitative proteomics, microbiome analysis, lipid profiling, and symbiont density measurements, we identified potential protein biomarkers that distinguish resilient corals from susceptible ones before a bleaching event. Our findings suggest that protein biosignatures, rather than pre-bleaching lipid stores, may provide a more accurate prediction of coral resilience, which has important implications for coral reef management and restoration strategies under climate change scenarios.

We have made significant changes to the manuscript in response to reviewer comments. In particular, we have (*note this is not an all inclusive list*):

1. Clarified terminology and provided more precise definitions of key concepts, such as “microbiome” and “biomarkers of resilience,” to ensure consistency and accuracy throughout the text.
2. Conducted additional microbiome functional prediction analyses using PICRUSt2 and MetaCyc *and* provided further discussion of the functional differences observed between resilient and susceptible coral microbiomes (new supplemental Figures 6a and b).
3. Streamlined the discussion to emphasize the broader implications of our findings and suggested future experimental directions, including pathogen susceptibility assays, metabolomics, and additional proteomic validations.
4. Enhanced the methods and results sections by adding more details on statistical analyses, experimental design (e.g., new Figs S1a, b), and sample sizes and types to improve reproducibility and transparency.

5. Addressed concerns regarding microbiome diversity metrics by incorporating a new figure (Figure 4d) to visually present differences in Faith's Phylogenetic Diversity between coral cohorts.
6. Adjusted the framing of our conclusions to ensure that claims about potential biomarkers are appropriately cautious and supported by the data.
7. Expanded the discussion on the logistical and financial considerations for developing rapid antibody-based tests from the proposed peptide biomarkers, providing a preliminary cost estimate and outlining the steps needed for broader application.

We believe that the revisions made in response to the reviewers' comments have greatly improved the quality and impact of our manuscript. The updated manuscript now provides a more comprehensive and nuanced understanding of the factors that contribute to coral resilience to thermal stress, offering valuable insights for conservation and management practices. We sincerely appreciate the reviewers' thoughtful input and believe that this collaborative effort has resulted in a more rigorous and impactful contribution to the field of coral biology and climate resilience research.

Thanks you for your dedication to your support and thoughtful feedback and consideration,

Brook L Nunn

Reviewer #1 (Remarks to the Author):

R1.1 The authors often use the term "microbiome" when apparently referring to only the bacterial community of the microbiome. This should be clarified throughout the text.

Author Response: Thank you for the comment. We have clarified this in lines 64-64, 120, and line 141 in the introduction. We have additionally added a clarification in the Results section line167-169.

Revised/new sentence: "As a holobiont, corals host a diverse community of organisms, including algal endosymbionts, and a bacterial, archaeal, and microbial eukaryotic microbiome that resides on and within the coral tissue, coral mucus, the coral gastric cavity and within endolithic skeleton."

Revised/new sentence: "Because our microbiome assays were based on 16S rRNA primers targeting bacteria and archaea, microbiome results describe the bacterial and archaeal portions of the microbiome (but not microbial eukaryotes)."

R1.2 The discussion needs to be condensed and streamlined significantly. The authors should try to explain the bigger picture story and implications of their results, as well as the experiments needed to verify their results (metabolomics and pathogen susceptibility assays).

Author Response: Thank you for your thoughtful suggestion. We have added the suggestion of additional experiments that could be conducted to further validate our findings. These are described below and have been added to the main text.

1. The finding that protein biosignatures are more informative than prior lipid stores in predicting bleaching resilience was surprising to us. This could be due to relative similarity of lipid stores pre-bleaching in our particular corals, or that prior energy storage is unpredictive of coral survival, even though bleached corals rely on stored lipids for survival. However, replication across taxa and conditions (e.g. including conditions where initial lipid stores vary) would be needed before this observation could be generalized.

2. To enhance the robustness of a future study and better account for biological variation, we suggest that additional *Montipora capitata* coral samples be collected in situ prior to the next natural heat-induced thermal stress event. Increasing the sample size and sampling of additional sites with a broader range of *Montipora capitata* genotypes would help to control for biological variability and increase confidence in the identification of biosignatures. These efforts should also incorporate proteomics, lipidomics, symbiont density measurements, and microbiome analyses to capture a more comprehensive understanding of the coral's physiological state. Additionally, the corals should be monitored after thermal bleaching to assess their resilience or susceptibility. We recognize the difficulty of this suggested study but stress the importance on moving forward on coral management.

3. To validate the current findings and biosignatures proposed, we recommend that further experiments be completed including pathogen susceptibility assays and metabolomics. In both cases, proteomics could be completed to validate both proposed assays by potentially linking all detected compounds to enzymes recognized as biosignatures of resilience.

Added sentences

"Further studies exploring the viral influence on downstream susceptibility, including tracking viral load with transmission electron microscopy to quantify and locate viral particles (i.e., in the endosymbionts or corals)."

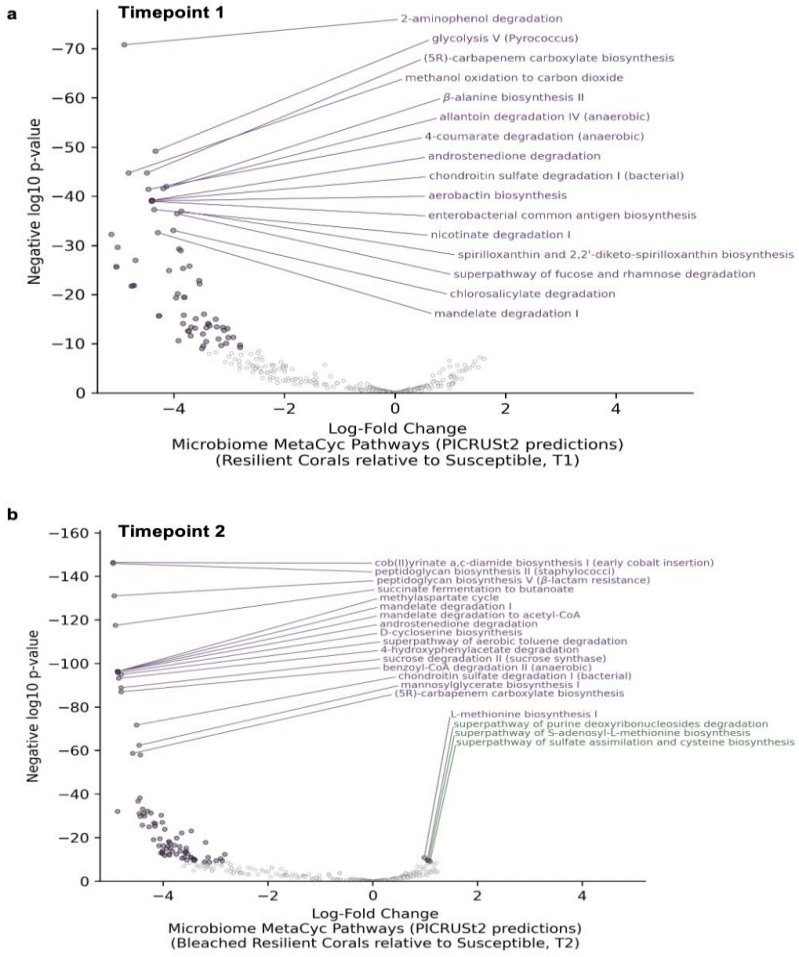
"The finding that protein biosignatures are more informative than prior lipid stores in predicting bleaching resilience was unexpected. This could be due to relative similarity of lipid stores pre-bleaching in these particular corals, or that prior energy storage is unpredictive of coral survival, even though proteomic analyses suggest bleached corals rely on stored lipids for survival. In either case, further studies across different coral species and experimental conditions, particularly those with greater variation in pre-bleaching lipid stores, are necessary to determine whether this pattern is broadly applicable."

End of concluding remarks: "To enhance the robustness of future studies and better account for biological variation, we recommend collecting additional *Montipora capitata* coral samples in situ prior to the next natural heat-induced thermal stress event. Increasing the sample size and including more sites with a broader range of *M. capitata* genotypes would help control for biological variability and increase confidence in identifying biosignatures. These efforts should incorporate proteomics, lipidomics, symbiont density measurements, microbiome analyses, and pathogen susceptibility assays, along with metabolomics across different coral sites and species, to provide a comprehensive understanding of coral physiological states. Additionally, monitoring coral resilience or susceptibility after thermal bleaching is essential for validating proposed biosignatures. While we acknowledge the logistical challenges of such studies, advancing these efforts is crucial for identifying reliable resilience markers and informing effective coral management strategies."

Regarding the recommendation to streamline the discussion, we recognize the concern that the discussion may overlap with the results. In response, we have removed some of the more obvious descriptions of enzymatic pathways from the discussion. However, we aimed to provide a detailed physiological pathway discussion to offer context on the changing metabolism, as we believe it enhances understanding for readers who may not be familiar with full metabolic pathway reconstructions. Our intention was to make these descriptions more accessible and broaden the readership. We hope to retain this portion of the manuscript, as we believe it adds clarity and value for a wider audience.

R1.3 Additional analyses (PICRUSt2 and METAGENassist) and data (depth of collection and outplanting to reef) should be added to the manuscript.

Author Response: Following your recommendation, we conducted a PICRUSt2 analysis of the microbiome data at T1 and T2 to understand the genetic potential of the microbiome in Resilient and Susceptible corals. There are some caveats to using PICRUSt in systems like corals that have been subject to much less intensive bacterial genome sequencing than others, e.g. the human gut. However, alone it can provide a useful general summary of the predicted functional capacities of microbes in the system. Two figures were generated to summarize these results and are included as supplementary figures S6a & 6b.



Although we have not exhaustively analyzed the predicted functional differences in Resilient corals, some pathways stand out among the most significantly enriched or depleted MetaCyc pathways. Antibiotic production (5R)-carbapenem carboxylate biosynthesis is lower in Resilient corals relative to Susceptible corals. It is possible microbial antibiotic production could play a role in the lower microbiome richness in Susceptible corals (e.g. due to microbe-microbe competition).

We also see lower predicted capacity for aerobactin biosynthesis in the microbiomes of Resilient corals at T1. Aerobactin is a siderophore used for iron scavenging, including by pathogens.

Intriguingly, we see a reduction in androstenedione degradation in Resilient corals relative to susceptible at T1. Androstenedione degradation has recently been proposed as a mechanism by which *Endozoicomonas* could influence host hormone signaling, especially under environmental stress (Oschenkukn et al., 2024). Notably, no consistent functional categories were enriched in Resilient corals' microbiomes at T1, while many categories were depleted relative to Susceptible corals. This may reflect the greater richness and flexibility of the

microbiomes in Resilient corals (i.e. fewer enriched categories are found because functional variance in Resilient corals is higher). In other studies, microbiomes with high inter-individual variability within a category often show this pattern (Zaneveld et al., 2016)

Added to Discussion "Additionally, the T_1R corals hosted significantly more diverse microbiomes (Fig. 4a). The predicted functional capacity of the resilient microbiome (i.e., PICRUSt2; Fig. S6) suggested the possibility that microbial antibiotic production in susceptible corals may contribute to the reduced microbiome richness observed in T_1S cohorts, potentially through mechanisms such as microbe-microbe competition. Higher microbiome diversity has previously been reported in more bleaching-resistant species, though this association does not always extend to bleaching susceptible or resilient conspecifics[68]. The resilient microbiome exclusively included the *Moraxellaceae* bacterial family. *Moraxellaceae* are associated with local wastewater and they are recognized to have high numbers of antibiotic resistance genes (ARGs)[69,70]. As the coral host's immune system is activated against pathogenic bacteria and releases antimicrobial defenses, the *Moraxellaceae* bacterial family's high number of ARGs may provide them with an advantage for long-term residence on the host. As *Moraxellaceae* has been found to be a common component of many shallow water coral microbiomes, these bacteria may be important in shaping a healthy coral holobiont[71]. The functional role of this bacterial family's unique genome in conferring resilience against bleaching is unknown, but the close association of *Moraxellaceae* with resilient *M. capitata* corals merits further research. Additionally, the PICRUSt2 analysis of coral microbiomes identified notable functional differences in resilient corals prior to bleaching. These results may reflect lower microbiome richness in susceptible corals prior to bleaching and point to higher functional variance in the microbiome of resilient corals, which could confer greater adaptability and an ecological advantage in the face of environmental stressors."

Reviewer #1 Specific Comments:

R1.4 L45: The sentence starting "Further, proteins identified…" is misleading. Firstly, "enriched" is a more accurate word than "amplified" when referring to proteins. Secondly, are you discussing differentially enriched proteins in this sentence? Did any proteins go the other direction?

Author Response: Thank you for the suggestions. We have changed the text in the abstract:

Revised/new sentence: "Furthermore, proteins significantly enriched before bleaching showed even greater abundance after bleaching, suggesting these pathways may be deterministic of a coral's fate when thermally bleached. "

R1.5 L68: Additional sources would be useful here, especially when the authors discuss reduced reproductive capacity.

Author Response: Thank you - we have added the following references

Hoegh-Guldberg, O. (1999). Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research, 50(8), 839–866.

Baird, A. H., & Marshall, P. A. (2002). Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Marine Ecology Progress Series, 237, 133–141.

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.

R1.6 L77-78: The connection between host specificity of microbiomes and microbiome disruption leading to host mortality needs to be expanded. As it stands, that connection does not seem obvious to the reader.

Author Response:

We have added the following text

Revised/new sentence: "Microbiome disruption can remove defensive benefits of normal coral microbiomes, which may include reducing pathogen infection success via multiple mechanisms (e.g. spatial occlusion and competition, direct predation, metabolic jamming, and quorum quenching (Krediet, Ritchie et al. 2013, Peixoto, Rosado et al. 2017, van de Water, Allemand et al. 2018, Beatty, Valayil et al. 2019)."

L80: Though the authors should note that gnotobiotic models for cnidarians are currently being developed (see Costa et al 2021: https://doi.org/10.3389/fmicb.2021.637834)

Author Response: Thanks for pointing out the study!- It is great to see advancements in this area. We have modified the text to read:

Revised/new sentence: "While gnotobiotic models are now being developed (Costa et al., 2021), fully unraveling the complex relationships between corals and their microbiomes remains a challenging task."

R1.7 L92: pre-bleaching once the stressor is removed? At this point, it might also be useful to define the terms resilience, resistance, and tolerance as they will be used throughout the text.

Author Response: We have clarified those definitions at your request in the text as follows:

From a range of papers, in particular the book by Salm(2005), we define the following: Bleaching ***tolerance*** refers to actual physiological properties of corals that allow them not to bleach in stressful conditions.

Resistance - The ability of an ecosystem to withstand disturbance without undergoing a phase shift or losing neither structure nor function (Odum, 1989). For example a coral reef's ability to withstand bleaching and mortality.

Resilience - The ability of a system to absorb or recover from disturbance and change, while maintaining its functions and services (Adapted from Carpenter et al, 2001). For example, a coral reef's ability to recover from a bleaching event. Or genetic markers allow insight into resistance and resilience of reef-building corals.

Revised/new sentence: "There are several possible contributing factors that may provide greater coral holobiont tolerance, defined as the physiological traits that enable corals to avoid bleaching under stressful conditions, as well as resilience, the ability of a coral reef system to recover from disturbances like bleaching while maintaining its functions. Some of these factors include: host- and/or symbiont- species[28-31], host genotype[32-37], or the microbial constituents of the holobiont's microbiome[38,39]. At the center of this complex equation is the host's metabolic capacity before, during, and after bleaching."

R1.8 L98-100: These are some of many possible contributing factors (e.g., life history, sex, etc). It might be worthwhile to mention that the factors you list are some of many possible factors

Author Response: Thanks for the suggestion, the sentence has been changed :

Revised/new sentence: "Some of these factors include host- and/or symbiont- species[28-31], host genotype[32-37], or the microbial constituents of the holobiont's microbiome[38,39]."

R1.9 L100: I am missing a connection between the sentence that ends in line 100 and the sentence that starts in line 100. The introduction of "immune function" seems sudden and not self-explanatory. Removing the parenthetical portion of the second sentence will help, as it is unnecessary (explained in the third sentence of the paragraph).

Author Response: We have removed the parenthetical portion of the second sentence as suggested to clarify the sentence**.**

Revised/new sentence: "At the center of this complex equation is the host's metabolic capacity before, during, and after bleaching. Without adequate carbon and nitrogen, the coral cannot maintain systems of cellular and tissue repair, retain, or reacquire symbionts, or fight off pathogens[40,41]."

R1.10 L104: Again, a connection is missing. Why is pre-bleaching molecular physiology key to determining resilience? It is not obvious here, as it could very well be something to do with the response to bleaching or the sym type hosted (e.g., what has been proposed with Durusdinium as low-photosynthate but high-stress resiliency, though this could also be debated).

Author Response: We have removed the phrase “pre-bleaching molecular physiology” and modified the sentence to improve clarity.

Revised/new sentence: “This suggests that the thermal threshold and physiological condition of the coral host may be the most important factors in determining resilience to bleaching stress and mortality.”

R1.11 L138: “contributing to bleaching resilience” is directional, but the differences in protein enrichment may not be actually contributing, just correlating or in response to the thermal stress/bleaching event

Author Response: Thanks, the reviewer has a valid point. We removed the phrase

Revised/new sentence: “Several proteins significantly differed between resilient and susceptible corals prior to experimental thermal stress.”

R1.12 L164: I’m not sure whether using the word “community composition” is accurate for the analyses that you’re performing, as you’re not doing metabarcoding (e.g., ITS2) to obtain a more total community composition makeup

Author Response: We have rephrased to say “Symbiodiniacea density and diversity”.

R1.12 L166: Is the symbiont density measured agnostic of symbiont type? As in, is it total symbiont density regardless of symbiont type?Because different sym types may be hosted at different densities which may affect host physiology/molecular physiology.

Author Response: Yes, symbiont densities include all the symbiont types, and yes, it is very likely that carbon acquisition and photosynthate translocation to the host can be influenced by different proportions of symbiont types. To clarify for the reader we have included “total” to refer to the symbiont density (L 35 and L183).

R1.13 L166-168: I wonder if the sym density decreases at the same rate for both sym types tested? Is there a way to measure this?

Author Response: Yes- there is a way to track this, however this was not the focus of the study and in this case we have no more sample left from the particular corals that we completed the proteomics on. We suggest the reviewer consider taking a look at Matsuda et al. 2023. This paper talks about how symbiont C and D grow differently under different temperature and light.

Other relevant papers: Bay et al. (2016) and the Cunning & Baker (2012).

Matsuda, S. B., Opalek, M. L., Ritson-Williams, R., Gates, R. D., & Cunning, R. (2023). Symbiont-mediated tradeoffs between growth and heat tolerance are modulated by light and temperature in the coral Montipora capitata. Coral Reefs, 42(6), 1385-1394.

(Cunning and Baker 2013)

(Bay, Doyle et al. 2016)

R1.14 L170: I wonder if the composition/domination changed throughout recovery

Author Response: We thank you for this question and are not focussed on this aspect for this paper past the timeframe mentioned. That said, Timmins Schiffman et al 2024 did track the symbiont composition over time front these corals and notes that there were no significant changes in the symbiont composition. Matsuda et al. 2023 explores a similar question and we encourage the reviewer to read that excellent study. Again, as we were sample-limited, this was not explored in this study.

Timmins-Schiffman, E., Duselis, E., Brown, T., Axworthy, J., Backstrom, C., Riffle, M., Dilworth, J., Kenkel, C., Rodrigues, L., Nunn, B. and Padilla-Gamiño, J., 2024. Reproductive resilience: pathways to gametogenic success in Montipora capitata after bleaching. *Scientific Reports*, *14*(1), p.27765.

R1.15 L249-251: In Figure 2f or in this section, it needs to be made clear that the Bleached (B) corals are all from the heat stress T2 and the Non-Bleached (NB) corals are from the control T1.

Author Response: Sorry for the misunderstanding- In the case for the lipids and the microbiome we had an additional cohort control carried through to T2 that was not bleached (as outlined in the methods) T_2NB refers to Timepoint 2, Non-Bleached corals.
We have tried to make it more clear by adding Timepoint 1 or 2 at the tops of the figure and in the text by rephrasing it to say:

Revised/new sentence: "For lipid analyses (see methods) an additional control cohort that was not bleached was followed from T_1 through to T_2 (as outlined in the methods). T_2NB, refers to lipids at T_2 from Non-Bleached corals. No significant difference in coral lipid content between T_1R and T_1S corals was found before the simulated thermal event (Dataset S1D-E; Fig. 2e). Coral lipid content varied significantly by bleaching status at T_2 (T_2B vs. T_2NB: p=0.00079; Fig. 2f) and resilience, or recovery capacity after bleaching (T_2R vs. T_2S: p=0.00095; Fig. 2e). Interaction of the two variables was also significant (p=0.044): susceptible corals experienced a decrease in mean lipid content by 44% after exposure to thermal stress (T_1S and T_2S), while resilient corals decreased by only 16% (T_1R and T_2R)."

R1.16 L285: Further analyses should be performed on the 16S data, for example PICRUSt2 and METAGENassist. These further analyses will help the authors investigate functional differences in the bacterial communities.

Author Response: Thank you for the suggestion- we have used PICRUST and included it in the manuscript. A discussion of its use is above (Reviewer Comment 1.3).

R1.17 L297-299: I wonder if there are some stats you can do on these differences/similarities in symbiont composition? Yes, both the resilient and susceptible populations had both D- and C-

dominated corals, but were the compositions indicative of anything? What about any background symbionts present?

Author Response:

We encourage the reviewer to check the methods section: "Symbiont community composition was determined to be Durusdinium-dominated, Cladocopium-dominated, or mixed-community based on the symbiont community composition in samples collected at T1 or from the non-bleached controls at T2, and Fisher's exact test was used to confirm that symbiont community type and resilience/susceptibility were independent. Further details found in SI Methods." (L718-724 in the manuscript)

We are not sure what they mean by "background symbionts" - if they are referring to symbiont types other than C and D, we can't capture those via qPCR as we only used primers for Cladocopium and Durusdinium.

If, however, they are wondering whether background levels of Durusdinium in the Cladocopium-dominated samples had any effect on resilience/susceptibility, we weren't able to do an analysis with a proportion of Durusdinium because of the missing data - unfortunately, the models weren't converging, which is why we opted for the categorization approach presented.

R1.18 L301: Is the statement "a common response to exogenous stressors" referencing translation?

Author Response: Here we are referring to previous papers that have revealed that when a system is stressed, commonly a more diverse suite of proteins are detected. (Dyhrman, Jenkins et al. 2012, Tilocca, Balmas et al. 2019)

In addition, here are a few of my papers that reveal this common trend in organisms faced with stressful conditions: (Nunn, Aker et al. 2009, Mattes, Nunn et al. 2013, Nunn, Faux et al. 2013, Poulson-Ellestad, Jones et al. 2014, Timmins-Schiffman, Coffey et al. 2014, Nunn, Slattery et al. 2015, Boyd, Dillingham et al. 2016, Timmins-Schiffman, Crandall et al. 2017, Mikan, Harvey et al. 2020, Glass, Ranjan et al. 2021, Mudge, Nunn et al. 2021, Pollara, Becker et al. 2021, Axworthy, Timmins-Schiffman et al. 2022, Gomes, Nunn et al. 2023)

R1.19 L310: I'm not sure if these differences "dictate" survival; it could be other things… life history? Reproductive capacity? Protein modifications? Also, the authors have not demonstrated that antiviral mechanisms are active (by pathogenic challenge), but have only demonstrated that some proteins/pathways are differentially regulated. In the coral immunity field, it is very common to conflate what has been qualified as molecular "immunity" pathways in higher order taxa with molecular immunity in more evolutionarily ancient metazoans. The authors should take care to make this clear in the text.

Author Response: Thanks for the suggestion- we have softened the statement to read “For the first time, this study examined how nutritional strategies, antiviral mechanisms, and microbiome diversity are associated with corals' capacity/likelihood to survive a bleaching event.”

R1.20 L319: This makes it sound like the susceptible corals aren’t performing heterotrophy… I think you’d have to do some behavioral assays and/or isotope tracking or something to make these claims

Author Response: To clarify the claim, this sentence, “Prior to bleaching, resilient corals utilize heterotrophic feeding and symbiont photosynthate. “ has been rephrased since we don’t have behavioral assay results. We have changed this sentence to be more clear, but also emphasize the results from the data.

Revised/new sentence: “Prior to bleaching, resilient corals, compared to the susceptible corals, have an increased relative abundance of enzymes that allow them to utilize heterotrophic feeding and symbiont photosynthate.”

R1.21 L346: Is there a citation for more diverse microbiomes supporting coral immune responses? Are you talking about molecular immunity or pathogen response/susceptibility?

Author Response: Thank you for the comment- we have added the following references and clarified the sentence..

(van Oppen and Blackall 2019, Mohamed, Ochsenkühn et al. 2023, Arriaga-Piñón, Aguayo-Leyva et al. 2024)

In the introduction statement to the discussion paragraph we wrote, “Prior to bleaching, the resilient corals appeared to prime anti-viral activity and exhibited a significantly more diverse microbiome, which likely supported their immune response.” The paragraph then discusses the statistically significant differences in protein responses associated with molecular immunity, which support the general claim regarding the resilience of corals. Later, the paragraph addresses the statistically significant findings about the microbiomes. In this section, we cannot determine whether the detected microbes are pathogens. However, the significant increase in the abundance of the *Moraxellaceae* bacterial family in the resilient microbiome aligns with previous research suggesting that this group of bacteria is associated with healthy corals. Relevant studies cited in the paper provide additional support for this argument.

R1.22 L402-403: The phrasing of this sentence makes it sound like you continued to measure Urease after T2. Did you? Does it increase at T2 compared to T1 in resilient corals?

Author Response: Sorry about this confusing statement. Although we do have urease concentration after T2, those results are published in Timmins Schiffman et al. 2024 and cover a less detailed analysis of the proteome (not focussed on those that survive vs those that die after bleaching. In that study in the corals that are resilient, urease abundances reach control levels by March after the experimental bleaching. That said, we are not including these results in this

paper. You are correct that the susceptible coral's URE1 levels were even higher at T2 relative to susceptible corals than they were at T1. This is a tricky sentence to write. We hope this clarifies it to the reader:

Revised/new sentence: "Following thermal bleaching, the abundance of urease (URE1) in the susceptible corals continued to increase, resulting in a greater divergence in URE1 levels between susceptible and resilient corals at T_2 compared to the pre-bleaching levels observed at T_1."

R1.23 L409: other types of microbiome analysis would help this claim (picrust2/metagenassist)

Author Response: Thank you for this suggestion. We examined the PICRUSt2 enriched MetaCyc categories to test if nitrogen fixation or scavenging showed clear differences. These analyses are included in Fig. S6 and Dataset 5 and discussed throughout the manuscript.

Note that we found no obvious differences in nitrogen fixation, but did find intriguing differences in microbial capacity for nitrogen scavenging. Specifically genes in the MetaCyc allantoin degradation pathway were strongly enriched in the predicted functional profiles for microbiomes of resilient corals. In plants, allantoin in roots is converted to allantoate and transported to leaves to provide nitrogen. Unlike humans and most metazoans, cnidarians have a ureidoglycolate lyase that can convert allantoin to glyoxylate.

R1.24 L433: The authors should be careful throughout the section around line 433 to not make claims that they have not demonstrated. Differences in pathogen immunity have not been directly demonstrated in the present study, and mucins may be involved in functions beyond pathogen-immunity.

Author Response: Thank you for this detailed comment. Lines 434, and 436-441 have been changed to make the claim less definitive.

line 434: "As mannose is recognized by lectin proteins in corals to identify pathogens and symbionts, the degradation of these mannose-based oligosaccharides **may** weaken physical associations of the host with symbionts and its microbiome[81,82]."

Revised/new sentence: " Increased presence of mucin proteins (*i.e.,* MUC4, Fig. 2c), a noted deterrent to pathogen colonization[83], and lectins, pathogen recognition proteins (e.g., TLEC2; Fig. 3, cluster 8) suggest that T_1 susceptible corals may also be more challenged by pathogens, further weakening their immune system prior to the bleaching event. Further studies on how increased mucin expression influences pathogen response in corals should be completed to test this hypothesis."

R1.25 L473: Did the authors observe that resilient corals activated heterotrophy and the susceptible ones didn't?

Author Response: The statements we have made in the manuscript about behavioral and physiological differences are all based on evidence from the proteome. When we detect changes in multiple proteins in a pathway, or in multiple linked pathways, we infer that there are macro-physiological changes associated with those molecular changes. We did not specifically measure feeding in this study. Lin 473 discusses different active metabolisms (based on observation of those proteins). The following sentences lines 475-493 discuss each enzyme

Revised/new sentence: "Multiple enzymes involved in endocytosis are increased in the T_2R cohort providing a heterotrophic avenue for carbon and nitrogen acquisition (Fig. 3, cluster 3). Increased abundance of two alpha-tubulin proteins (TUBA), vacuolar sorting endocytic protein (VPS4), dynamines and coatamers (COPG, COPB2) imply increased endocytic activity of particles, such as pathogens or food[80,88]. Significantly increased abundance of lysosome-associated membrane protein (LAMP) may indicate that symbiont engulfment and degradation is an additional potential source of nutrition for resilient corals post bleaching[79]. Significantly increased peptide degradation enzymes in the T_2R cohort included glutamyl amino peptidase (ENPEP, (Fig. 3, cluster 3), which cleaves acidic amino acids from the N-terminus of peptides for subsequent degradation to enhance cellular growth. T_2R also significantly increased a vitellogenic carboxypeptidase (CPVL), a protein involved in the degradation of yolk proteins. This could be a biomarker of decreased yolk provisioning to developing gametes in bleached resilient corals since this species only transfers autotrophic carbon to its gametes (Padilla-Gamiño et al., 2012a; Rodrigues & Padilla-Gamiño 2022; Jaffe et al., 2023). Abundant protease/peptidases (Fig. 3, cluster 3) and lipases (*e.g.*, triacylglycerol lipase PNLIP; Fig. 2d) in resilient corals can break the bonds of macromolecular complexes to generate mobile small molecules that can be recycled or further degraded for energy. In support of these findings suggesting adequate nutritional resources were available in the resilient cohort post-bleaching, the increased abundance of transketolase (TKT) in T_2R may indicate a higher abundance of thiamine (vitamin B1) compared to T_2S[89,90]."

R1.26 L506: This sentence needs to be changed. The authors don't know whether T2R did this because they don't have pathogenic data; the immune system is complicated and these proteins may be doing something completely different

Author Response: Thank you for your suggestion, we have reworded it to clarify the findings and suggested interpretation.

Revised/new sentence: "Proteomics analyses also suggest T_2R also launched an antiviral campaign during thermal stress to assist the immune system. Cyanovirin protein (CVNH) was detected in resilient corals at T_1 and T_2 at three-fold higher abundance compared to susceptible corals (Fig. 3, cluster 1). Although we do not believe this is the only mode of protection for the resilient cohort, high production of this protein could increase the resilience of these corals after bleaching events when they are simultaneously coping with multiple stressors."

R1.27 L549: It would make more sense to the storyline of the discussion if there was an "immune" section

Author Response: Thank you for this suggestion, but immune pathways are not our expertise and coral immunity is not well understood, we did not want to overstate our findings. We hope that our findings will provide groundwork for immunity experts to test hypotheses in future work.

R1.28 L565: be more specific with what you mean with "protein" and "microbiome" here
Author Response: Thanks for your comment. We have clarified the language to read

Revised/new sentence: "We identified two key biological factors that distinguish resilient from susceptible corals before the advent of thermal bleaching that forecast their long-term health: protein abundances (Fig 2a,c) and microbiome diversity (Fig. 4a)."

R1.29 L569-on: I don't think you can say for sure that these are biomarkers of resilience with only n=12
Author Response: We fully agree that the biomarkers need further validation and have read through the manuscript to make sure that the wording is not overstating the findings. In each case where we mention protein biomarkers we made sure it says "potential protein biomarkers".

We added to Concluding Remarks: " To enhance the robustness of future studies and better account for biological variation, we recommend collecting additional *Montipora capitata* coral samples in situ prior to the next natural heat-induced thermal stress event. Increasing the sample size and including more sites with a broader range of *M. capitata* genotypes would help control for biological variability and increase confidence in identifying biosignatures. These efforts should incorporate proteomics, lipidomics, symbiont density measurements, microbiome analyses, and pathogen susceptibility assays, along with metabolomics across different coral sites and species, to provide a comprehensive understanding of coral physiological states. Additionally, monitoring coral resilience or susceptibility after thermal bleaching is essential for validating proposed biosignatures. While we acknowledge the logistical challenges of such studies, advancing these efforts is crucial for identifying reliable resilience markers and informing effective coral management strategies."

R1.30 L577: how would these things be developed?

Author Response: To develop a MS based protein assay to determine if a coral will survive a bleaching event, the identified predictive peptide biomarkers must first be tested on corals from diverse locations and environmental conditions and with large sample sizes. Completing targeted mass spectrometry based proteomics on a range of samples with a fast MS assay would allow you to determine the success of the assay. This step is critical to confirm that these biomarkers are universally reliable indicators of bleaching resilience across various coral species and habitats. While we have responded to the reviewer, we are not going to include a detailed description of how to develop peptide assays in this manuscript as it would consume a about a page of text.

R1.31 L583: has the rice grain size thing been verified?

Author Response: Yes- I can personally validate this. For proteomic mass spectrometry, you need ~1ug total protein per MS analysis. I have been performing high throughput proteomics for > 25 years on a range of samples from the marine environment. For starting material, we request ~20 micrograms of total protein (for the case of a bacterial cell pellet, that is ~10^9 cells) because of tube transfer and ease of handling. Automated technology is, however, allowing us to do less. A rice grain size pellet of tissue, such as coral, has 1000-2000 micrograms of protein.

Our group has now completed > 10 studies on coral and we use less than a rice grain size as our initial sample. After we have lysed the cells and determined the total amount of protein in the lysate- we subsample 50 µg for downstream preparations.

(Petrou, Nunn et al. 2021, Tisthammer, Timmins-Schiffman et al. 2021, Axworthy, Timmins-Schiffman et al. 2022, Jaffe, Padilla-Gamiño et al. 2023, Padilla-Gamino, Timmins-Schiffman et al. 2024, Timmins-Schiffman, Duselis et al. 2024) (and 4 are in the publication pipeline now as either accepted or in review manuscripts)

R1.32 L602: depths of collection?

Author Response: Colonies were collected in a patch reef in Kaneohe Bay at 1.5-2 m depth. I have included the text.

Revised/new sentence:". Seventy-four *Montipora capitata* (approximately 30 cm in diameter) were collected from at 1.5-2 m depth Moku O Lo'e island (patch reef) located in Kāne'ohe Bay, O'ahu, Hawai'i (21.428°N, 157.792°W) in August 2017."

R1.33 L606-610: Forgive me if I am missing something, but how does 2 deg every day for four days only take you from 28 to 30?

Author Response: Thank you for highlighting this mistake. We have replaced the text with:

Revised/new sentence: "To reach the 30°C temperature target for the bleaching treatment, experimental tank temperatures were increased around 0.5-0.6°C per day for four days using a 600 W Titanium Aquarium Heater (Bulk Reef Supply, United States)." (lines 672).

R1.34 L618: depth?

Author Response: Coral racks were also located at 1.5-2 m depth.

Revised/new sentence: "After thermal exposure, all corals (bleached and not bleached) were placed on racks at 1.5-2 m depth off HIMB (where they were originally collected from) to monitor survival and physiological recovery in situ for eight months."

R1.35 L643-645 ish: Cite something for these symbiont types? Its2 seq?

Author Response: We have included two additional references in this sentence for the reviewer. These earlier publications demonstrate that M. capitata has dominant clades of Durusdinium and Cladocopium, as identified using ITS2 sequencing (Stat, Bird et al. 2011, Padilla-Gamiño, Pochon et al. 2012)

R1.36 L687-689: The development of the p-values needs to be clarified. Did the authors assign these p values themselves or are they from statistics?

Author Response: The p-values are directly related to the QPROT-QSPEC package cited.

We clarified the text:

Revised/new sentence: "Differential relative protein abundances for resilient vs. susceptible corals and corresponding p-values were determined for each timepoint (T_1 and T_2) using the QPROT-QSPEC package[108](Dataset S2G-H)."

R1.37 L725: Picrust and metageneassist
Author Response: We have added new sections in response to this request.

Reviewer #2 (Remarks to the Author):

The manuscript presented by Nunn and collaborators represents a complete insight into understanding why the differences in the recovery capacity of different coral colonies of the same species are distributed in a particular site. This is of special interest since it is assumed that at the species level, corals obtain the same tolerance to stressors; however, at the time of a bleaching event, the same species may have different responses. The manuscript is very well written, and the information is pertinent and flows within the text. Within the manuscript, the goal, the methods used (both experimental and laboratory), the results, and the discussion that not only covers the results but also seeks a relationship between the markers that were used are clear. Therefore, my comments are minimal, and the manuscript can be considered for publication after a minor review.

Author Response: Thank you for your thoughtful and positive feedback on our manuscript. We are delighted that you found the study well-written and the information clear and relevant. Your recognition of the importance of understanding recovery capacity differences among coral colonies and how our findings contribute to this field is deeply appreciated.

We value your insights and will carefully address any additional comments or suggestions you may have during the minor review process to further strengthen the manuscript. Thank you again for your kind and encouraging words.

R2.1 Line 61. the thermal threshold is not only for a given coral species, but also for the given regional and local conditions.

Author Response: Thank you for this suggestion we have modified the text: Correct, we should modify the text. I suggest:

Revised/new sentence: "When water temperatures exceed local thermal thresholds, the association between the coral host and its endosymbiotic algae, Symbiodiniaceae, breaks down."

R2.2 Line 104. The bleaching response is the visual evidence of the stress, regardless of the physiological response; therefore, it would not be more accurate the therm tolerance threshold or resistance threshold.

Author Response: We agree with your suggestion and have changed the text to read:

Revised/new sentence: "This suggests that the thermal threshold and physiological condition of the coral host may be the most important factors in determining resilience to bleaching stress and mortality."

R2.3 Line 141. Is the term rejection accurately used (in general along the manuscript)? the corals already have "accepted" the symbiont, so they are trying to discard the already accepted symbionts.

Author Response: Thank you for the clarifications, we have modified the statement to say "symbiont disassociation" instead of "symbiont rejection" in line 144.

Revised/new sentence: "These resilience-associated proteins suggest nutritional and metabolic strategies may underpin the ability to survive bleaching. The proteome also revealed evidence for symbiont disassociation, antiviral activity, enhanced immune response to pathogens, and carbon and nitrogen pathways exclusive to resilient corals."

R2.4 Line 151. Clarify the ambient temperature (even if it is included in the materials and methods section.

Author Response: We revised the text in lines to include ambient temperature mean and standard deviation: 27.7C ± 0.6 (max=28.8, min=25.8 for the duration of the experiment)

Revised/new sentence: "In September 2017, seventy-four *Montipora capitata* were tagged and collected. Corals were cut in half, acclimated in tanks for two weeks, and sampled (T_1). Then, each coral half was either maintained at ambient temperatures (27.7^{o}C ± 0.6, range = 25.8 - 28.8 ^{o}C) for the duration of the experiment (Fig. 1a) or gradually exposed to increasing water temperatures to reach 30^{o}C."

R2.5 Line 152. Does the 30°C have no fluctuation at all?? why all the temperature data does not include standard error or standard deviation??

Author Response: Thank you for finding that oversight. We have included the ranges and standard deviations as requested and apologize they were not mentioned before.

Revised/new sentence: "Coral fragments in the high-temperature treatment were held at 30°C ± 0.5 °C (range = 28.9 - 31 °C) for three weeks to simulate a thermal bleaching event."

R2.6 Line 289. thermal-induced or trigger bleaching response?

Author Response: The sentence as written is: "Our multidisciplinary approach provides unprecedented biochemical details that link metabolism to expressed physiology in resilient and susceptible Montipora capitata before and after thermal-induced bleaching." We focused exclusively on thermal bleaching responses throughout the paper, as this aligns with the study's objectives. Expanding the discussion to include other potential factors that might induce bleaching would require a more complex analysis and was beyond the scope of this project. We appreciate the reviewer's insight and recognize the importance of these additional factors, which could be explored in future research.

R2.7 Line 344. because it is considered that the process of energy is being stored; It has been shown that organisms that are subject to stressful conditions and even more so that have the ability to cope, are characterized by a higher metabolic rate (See Seibel and Drazen 2007), so that energy can be used as part of the energy I would say,

Author Response: Thank you for your insightful comment regarding Line 344. We agree that organisms under stress, particularly those capable of coping, often exhibit higher metabolic rates to support immediate physiological demands and adaptive responses, as highlighted in the referenced study. The sentence in question states, "Additional evidence, such as increased abundance of pyruvate dehydrogenase (PDHa; Fig. 2c) and multiple acetyl-CoA transferases support increased cellular respiration and the potential to store excess energy in resilient corals." While our findings suggest the potential for energy storage, we acknowledge that this increased metabolic activity may also directly support physiological processes critical for resilience. We will revise the sentence to reflect this broader interpretation and cite the suggested reference to provide additional context.

Revised/new sentence: "Additional evidence, such as increased abundance of pyruvate dehydrogenase (PDHa; Fig. 2c) and multiple acetyl-CoA transferases, supports increased cellular respiration in resilient corals, which may contribute to both immediate physiological demands and the potential to store excess energy (Seibel and Drazen 2007)."

R2.8 Line 345. If the antiviral response is triggered by the thermal stress, the corals should be less susceptible to diseases; however on the contrary it has been recorded that during the bleaching response corals are more susceptible to be infected (e.g. Croquer 2009, Weill et al. 2009, Brand and MCManus 2009; Miller et al 2009). So, it has not been explored whether these enzymes may have other functionalities as metabolic repairers or any homeostasis balance pathway rather than anti-virals.

Author Response: We suspect that all corals bleached in our study would be more susceptible to disease, as has been shown in the studies you mention. It is possible, however, that the R corals are less susceptible when compared to the S corals. That hypothesis would require further testing and disease trials were not part of our objective.

Protein functional annotation is a problematic process, if looked at too carefully. As researchers on non-model systems, we rely largely on sequence homology with better characterized organisms to name and categorize proteins in corals, copepods, bacteria, etc. We hope that caveat is understood by most consumers of non-model 'omics articles. So, yes, it is possible that these proteins have different functions in corals than they do in the organism in which they were characterized. But immune response proteins are also likely to be conserved in sequence and function across taxa, as the basic mechanisms of immunity seem to be the same across taxa.

R2.9 Line 487. Review the citation format.

Author Response: Fixed. thanks

Lines 559-574. It is interesting that these markers want to be applied for management and conservation; However, it would be important to describe the limitations, mainly in cost, that this would have, what would be a possible proposal.

Author Response: The primary limitation, as we see it (as experts in proteomics), is replicating these experiments on a larger scale across different coral species, reef systems, and environmental conditions to confirm the reliability of the proposed peptide biomarkers. This point is now included in the Concluding Remarks section of the manuscript.

As far as generating a rapid antibody-based test, we anticipate that a specialty lab or biotech group could achieve this relatively easily once the peptides are validated. My back-of-the-envelope calculation would be a proposal for an initial development cost of approximately $500,000 to $1,000,000, which would include peptide synthesis, generation of monoclonal antibodies, and preliminary validation assays and support for the research. Scaling production for broad use in the field would add additional costs but could be reduced through partnerships with diagnostic companies. Once established, however, the per-test cost could be kept relatively low (approximately $10 to $20 per test), making it feasible for reef monitoring programs and conservation efforts. Importantly, initial validation would also require testing across a wide range

of coral populations to ensure robustness of the biomarkers under different environmental and stress conditions.

Although I understand the reviewers concerns, I am not including these details in the manuscript as I do not feel that is the goal of the research– a follow up review article could be generated for this type of discussion.

R2.10 Line 606. Is the temperature constant and without any variation?

Author Response: Thank you for finding this mistake. We have included more information on the temperature variation during the first two weeks of acclimation.

Revised/new sentence: "Corals were brought to shore and acclimated in flow-through outdoor tanks at the Hawaii Institute of Marine Biology (HIMB) for two weeks at ambient conditions (27± 0.4 °C, max=28.1, min=25.7)."

R2.11 Line 609. Again, Since the research is based on the response to temperature regimes, the variation that could exist both during the acclimatization and stress periods must be described.

Author Response: Thank you for noticing this- we have revised the **Coral Collection and Experiment** section to include more details about the temperature in addition to a new supplemental figure to show the temperature though time in each tank (Fig S1).

Revised Section :

"**Coral Collection and Experiment.** Seventy-four *Montipora capitata* (approximately 30 cm in diameter) were collected from at 1.5 - 2 m depth Moku O Lo'e island (patch reef) located in Kāne'ohe Bay, O'ahu, Hawai'i (21.428°N, 157.792°W) in August 2017. Corals were brought to shore and acclimated in flow-through outdoor tanks at the Hawaii Institute of Marine Biology (HIMB) for two weeks at ambient conditions (27± 0.4 °C, max=28.1, min=25.7). At the time of collection, corals were divided into two pieces to compare physiological performance for the same genets with/without exposure to thermal stress (Fig. 1 a, b). In September, one half of each coral was exposed to warmer water temperatures to simulate a natural thermal bleaching event[53]. To reach the 30°C temperature target for the bleaching treatment, experimental tank temperatures were increased by ~ 0.5°C per day for four days using a 600 W Titanium Aquarium Heater (Bulk Reef Supply, United States). Throughout the bleaching experiment, the control tanks (n = 3) had a temperature of 28 ± 0.6°C (range: 26.5-30.7°C), and the high-temperature tanks (n = 3) had a temperature of 30 ± 0.5°C (range: 28.9–31°C)(Fig. S1a).

Corals were rotated once a week among the same treatment tanks to minimize tank effects. For the bleaching experiment, *M. capitata* were kept at this elevated temperature for three weeks to induce bleaching in all corals. After three weeks, the tank temperature was lowered, following the previously described rate, back to ambient temperature (27-28°C) and another set

of tissue subsamples were taken (temperature data: Dataset S1B). The control experiment coral halves (T_2NB) not exposed to thermal stress remained at 28°C and were rotated each week among tanks of the same treatment to minimize tank effects[53] and collected at the same time as the bleaching experiment (details below). Mean daytime photosynthetic active radiation (PAR) levels in the tanks were 584 µmol photons m^{-2} s^{-1}, and mean PAR at 12:00 was 1,249 µmol photons m^{-2} s^{-1} , measured using a waterproof PAR logger (Odyssey, Dataflow Systems Limited, NZ; Fig. S1b). These irradiance values were very similar to values in the field where the coral colonies were located(Padilla-Gamino, Bidigare et al. 2013). For more information on the experimental setup see (Axworthy, Timmins-Schiffman et al. 2022, Timmins-Schiffman, Duselis et al. 2024). After thermal exposure, all corals (bleached and not bleached) were placed on racks at 1.5 - 2 m depth off HIMB (where they were originally collected from) to monitor survival and physiological recovery in situ for eight months."

Reviewer #3 (Remarks to the Author):

Summary
The manuscript "Resilience in a time of stress: revealing the molecular underpinnings of coral survival following bleaching events" by B Nunn and colleagues investigates the mechanisms underlying resilience and susceptibility of the coral Montipora capitata following an experimental bleaching event. The authors generated mass-spectrometry based proteomic data, along with characterizations of coral microbiomes, total lipids, symbiont density, and symbiont genera. The authors identify microbiome diversity differences in resilient and susceptible corals prior to bleaching, and identify differentially abundant proteins relevant to the holobiont stress response. Additionally, the authors link protein abundance prior to bleaching with abundance after bleaching, with potential importance for predicting coral resilience ahead of bleaching.

This manuscript represents a significant research effort and contains interesting data (particularly integrating proteomics with other more often measured phenotypes) that are relevant to the field of coral biology. However, I do have several points of concern that I feel need to be addressed before this manuscript can be published. Primarily, I feel that the manuscript does not contain sufficient detail in the methods and results to fully interpret or facilitate reproduction of the data presented. I highlight specific lines in my detailed comments below, but in general the results are missing important statistical analyses and the methods need more detail throughout. Specifically, for the microbiome analysis, I found what seem to be discrepancies between the supplemental information and the main manuscript, and ultimately feel that all of the details presented in the supplement are necessary to include in the main manuscript. Additionally, the authors make broad claims in the abstract and introduction about presenting a "multi-factor approach to identify whole-organism biomarkers of resilience". However, from my perspective the only convincing biomarkers you present are the protein data, as creating biomarkers from overall differences in microbiome diversity would be challenging (see specific comment below on the discussion). Additionally, the authors highlight the importance of microbiome diversity throughout the manuscript, and cite Figure 4a as illustrating

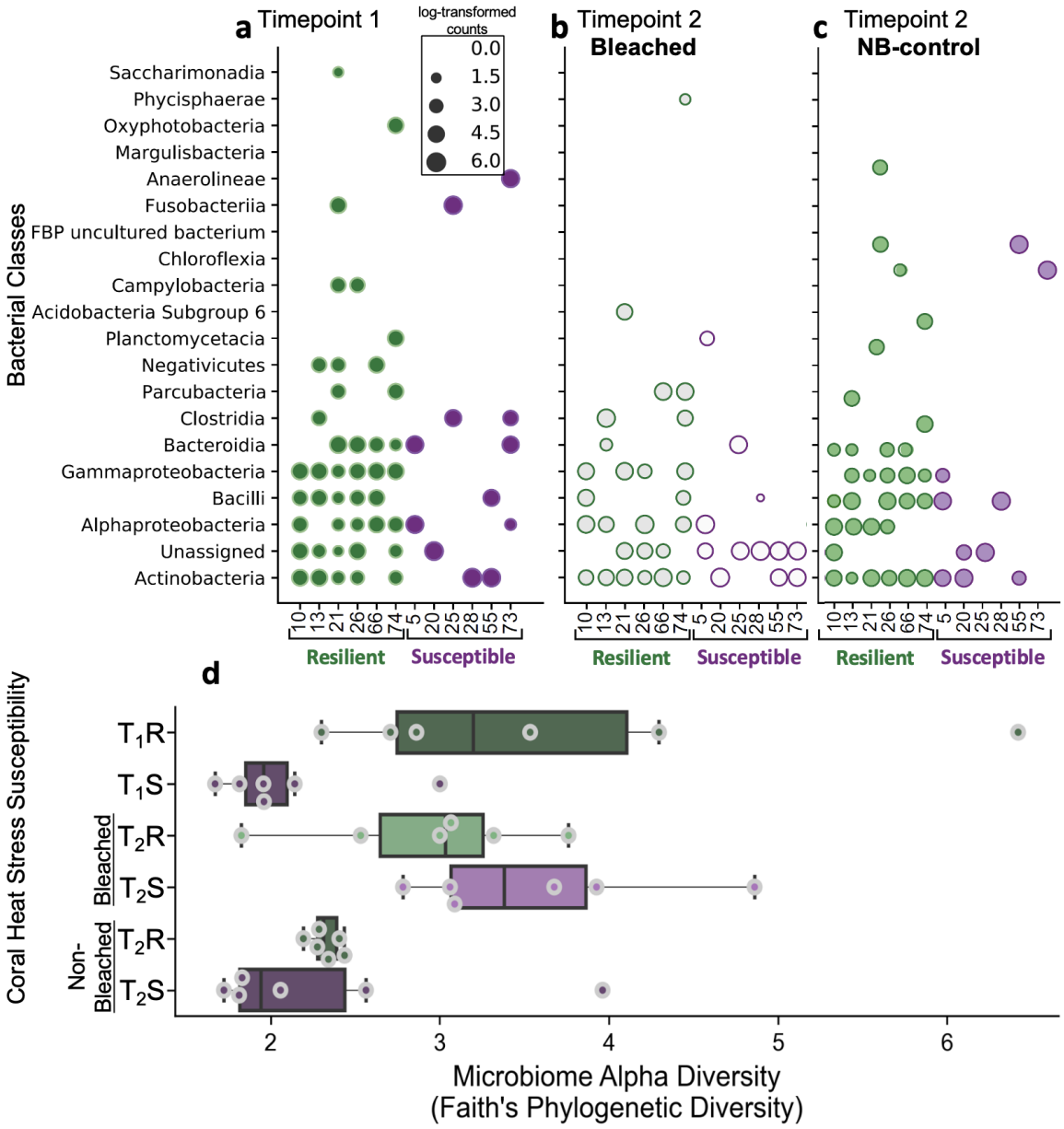
such differences in microbiome diversity at the end of the discussion, but from my understanding of the legend and the data included Figure 4a does not seem to include any diversity metric but rather transformed counts of specific taxa. Faith's PD is presented in the results but not in any figure that I found, which in my view would be the more appropriate visual representation of these important microbiome diversity differences. I suggest being more specific in the claims about identifying protein biomarkers of resilience throughout the manuscript or adding in a more thorough presentation of the differences in microbiome diversity.

For these main reasons, along with my detailed comments below, I recommend that this manuscript be accepted following major revisions. In addition to my main points highlighted above, I have included line-by-line suggestions below that should be addressed before publication.

Thank you very much for your thorough review and thoughtful comments. We greatly appreciate your recognition of the significance of our research and the integration of proteomics with other phenotypic data. We will carefully address each of your concerns, including providing additional detail in the methods and results sections (see tracked changes to see all additions), clarifying statistical analyses, and ensuring consistency between the main manuscript and the supplemental information.

We have revised our claims about identifying biomarkers to better reflect the strength of the protein data, and present microbiome diversity more clearly, including adjusting the figure presentations as suggested (see below Figure 4d has been added in response to Reviewer 3's comments). Your feedback has been invaluable.

Figure 4



Abstract

L37: “16S rRNA of the microbiome” seems an incomplete way to describe this method, I suggest “sequencing of 16S rRNA to characterize coral microbiomes” or something similar.

Author Response: Thanks for the suggestion, the exact wording was replaced as suggested:

Revised Sentence: "Using an integrated systems-biology-approach that included quantitative proteomics, 16S rRNA sequencing to characterize the coral microbiome, total coral lipids, symbiont community composition and total symbiont density, we explored molecular-level mechanisms of tolerance in corals pre- and post-bleaching."

L39: Specify 'protein biomarkers' here
Author Response: Thanks. We have modified it to read:

Revised/new sentence: "Our results have important implications for the future of reefs, revealing molecular factors necessary for surviving thermally-induced bleaching events and identifying promising diagnostic protein biomarkers for coral reef management and restoration."

Introduction
L68: I suggest adding a citation here for the statement about widespread mortality.
Author Response:

The expulsion of Symbiodiniaceae during bleaching events metabolically compromises the host[11], leading to reduced physiological performance, reduced reproductive capacity (Szmant and Gassman 1990, Hoegh-Guldberg 1999, Baird and Marshall 2002), and oftencan lead to widespread mortality (Boilard, Dubé et al. 2020, Helgoe, Davy et al. 2024)

L89-92: Please add citations for both of these sentences here.
Author Response:

Coral death due to the interconnected physiological and microbiological consequences of bleaching events can lead to total community collapse if mortality is widespread(Baker, Glynn et al. 2008, Weis 2008)

However, some coral species, and individuals within species, are resilient to the effects of bleaching and can recover (West and Salm 2003)

L109: Please add a citation for this statement.
Author Response:

The interactions between an organism and its environment are complex and depend upon ecological and evolutionary history. Corals in Kāneo'he Bay, O'ahu, Hawai'i have endured an increased frequency of bleaching events since 1996, similar to worldwide trends (Bahr, Jokiel et al. 2015, Eakin, Sweatman et al. 2019, Ritson-Williams and Gates 2020)

L135: It is unclear what "semi-quantitative proteomics" means here, I suggest defining it either here or in the methods to highlight how it differs from a fully quantitative proteomic method.

Author Response: We have changed the text to be more clear to this readership:

Revised/new sentence: "Comparison of resilient vs. susceptible *M. capitata* using mass spectrometry based proteomics allowed for the generation of metabolic maps of abundant enzymes and outlined the coral's energetic priorities that may confer resilience in a changing climate."

General Comment: You mention that you outline a multi-factor approach to identify whole-organism biomarkers of resilience, but after reading through the manuscript in its entirety I do not agree that you accomplish this. You identify protein biomarkers that are potentially related to resilience, but no other factors are included in my opinion. I suggest re-phrasing to be more specific about protein biomarkers.

Author Response: Thanks for the suggestion. We have modified the sentence to read:

Revised/new sentence: "Last, we identify biomarkers in corals that have the potential to predict survivorship during future bleaching events, the linchpin to coral management, propagation efforts, and restoration success."

Results
L150: Were these 74 Montipora capitata confirmed to be genetically unique individuals? Did you check for clones in your dataset? Same comment for the 6 resilient and 6 susceptible colonies that you select – were you able to confirm that they were unique genotypes?

Author Response: We did not confirm they were genetically unique individuals.
Individual colonies were collected more than 3 m apart and although no genetic analyses were conducted, they were considered to be different genets.
Caruso et al. (2022) confirmed high genetic diversity of Montipora capitata at our study site and showed that clonality is rare and mostly restricted to habitats conducive to fragmentation by wave energy.

Caruso, C., Rocha de Souza, M., Ruiz- Jones, L., Conetta, D., Hancock, J., Hobbs, C., ... & Drury, C. (2022). Genetic patterns in Montipora capitata across an environmental mosaic in Kāne'ohe Bay, O'ahu, Hawai'i. Molecular Ecology, 31(20), 5201-5213.

L150-153: It is unclear here whether you included a genotype control in this experiment, but from looking at the methods it does seem like that was the case. I think that is worth specifically mentioning here.

Author Response:

Yes, we included a genotype control in our experiment.

We have modified the text in lines 153-156.

"In September 2017, seventy-four *Montipora capitata* were tagged and collected. Corals were cut in half, acclimated in tanks for two weeks, and sampled (T_1). Then, each coral half was either maintained at ambient temperatures (27.7°C ± 0.6, range = 25.8 - 28.8 °C) for the duration of the experiment (Fig. 1a) or gradually exposed to increasing water temperatures to reach 30°C. Coral fragments in the high-temperature treatment were held at 30°C ± 0.5 °C (range = 28.9 - 31 °C) for three weeks to simulate a thermal bleaching event (Fig. 1b). After the thermal stress, experimental tanks were returned to ambient temperatures over four days. After 24 hours at the ambient temperature, corals were sampled again (T_2), placed on racks in the natural environment, and monitored for long-term survival and recovery for eight months (Figs. 1a, b and S1)."

L158: Please define 'susceptible' as 'S' here, similar to what you do on line 159 for resilient corals.

Author Response: Thanks- completed/fixed.

L166-172: You are missing p-values for this entire paragraph, please add in results of a statistical analysis to compare the symbiont density values across treatments and time.

Author Response: We have gone through the text to include p-values. Note tharp-values were calculated for each reported analysis. All p-values are also present in supplemental datasets.

Revised/new sentence: "At T_1, no significant difference in total symbiont density was detected between the resilient and susceptible corals (p = 1, Fig. 1b, Dataset S1a). During the thermal stress event, symbiont density decreased in both cohorts at the same rate; within three months after T_2, the resilient cohort returned to pre-bleaching Symbiodiniaceae density. Corals were classified as being *Cladocopium*- or *Durusdinium*-dominated or having mixed communities (see Supplemental Methods; Table S1). Notably, there were representatives of each community type in both the resilient and susceptible categories (Fig. 1c)."

L200-201: Here you mention that you only report p-values for significant results, but I do not see any p-values in this paragraph. Please add them. Also, I do not think this is an appropriate approach for the other physiology metrics that you present (see earlier comment L166-172), and all p-values should be included in the results.

Author Response:

The sentence being referred to is : "To elucidate complete metabolic pathways being preferentially utilized by either the resilient or susceptible corals at the two time points, significant differential abundances of proteins were calculated (Fig. 2c, d; significance only reported when $p \leq 0.05$; Dataset S2G-H)."

For proteomics results it is standard to define a p-value threshold to report all downstream significant differences. These p-value cutoffs can vary depending on the goal of the project and be reported at the peptide level or the protein level. To make the manuscript easier to read, the

standard approach in my field is to report all pvalues in the datasets for users to explore if needed.

L221-222: I'm not sure if I am missing it, but I'm unclear where you previously mention a protein involved in symbiont degradation. Please clarify.

Author Response:I have removed the phrase that was a remnant of prior text, as it is no longer relevant in the current draft.

L227-240: Please include a couple of extra references to Figure 3, those are missing from the second part of this paragraph.

Author Response: Extra references are now included throughout the paragraph.

L248-249: Again, missing p-values for this statement, please add them.

Author Response: We have added the p-values into the text.

Revised/new sentence: "Coral lipid content varied significantly by bleaching status at T2 (T2B vs. T2NB: p=0.00079; Fig. 2f) and resilience, or recovery capacity after bleaching (T2R vs. T2S: p=0.00095; Fig. 2e)."

L251: Be more explicit about the two variables here, I am pretty sure you mean the interaction between bleaching status and time point but will be helpful to clarify for the reader.

Author Response: Thanks for the suggestion we have modified the sentence as suggested.

Revised/new sentence: "Interaction of the two variables (i.e., bleaching status and time point) was also significant (p=0.044): susceptible corals experienced a decrease in mean lipid content by 44% after exposure to thermal stress (T1S and T2S), while resilient corals decreased by only 16% (T1R and T2R)."

L255-266: Are there figures to cite for these comparisons of Faith's PD? I'm unclear of where to find these data, which doesn't seem appropriate since you make large claims about the importance of microbiome diversity and its role in coral resilience. You cite Figure 4a in the final paragraph of the discussion to highlight differences in microbiome diversity, but this seems to just be abundance (i.e., counts) of a few taxa, and not representative of the metrics of diversity that you highlight in the results. I think that presenting these data in a figure would be most appropriate with the attention that you pay to the importance of microbiome diversity.

Author Response: Thank you for the suggestion and sorry for the confusion. We have made a new figure (Fig. 4d; inserted above in the response text) and incorporated that into the manuscript.

Discussion
L296-299: Instead of making this qualitative statement, I think you should include a statistical test to specify this point. For example, you could run a linear model relating the proportion of Durusdinum hosted by an individual pre-bleaching to mortality post-bleaching

Author Response:

We thank the reviewer for their thoughtful feedback and attention to detail. We conducted a simple logistic regression analysis to assess whether the proportion of Durusdinium symbionts influenced survival rates and have added that to the manuscript and github repository.

Results section: "A logistic regression analysis was conducted to assess whether the proportion of Durusdinium symbionts influenced survival rates. The analysis indicated a positive association between the proportion of Durusdinium symbionts and post-bleaching mortality (coefficient = 2.38); however, this relationship was not statistically significant (*p=0.342*). Additionally, the model's intercept was not statistically significant (*p=0.345*), suggesting that baseline mortality was not strongly predicted in this dataset (Dataset S1B)."

Discussion Section: "However, corals with *Durusdinium*- and *Cladocopium*-dominated symbiont communities were represented in both the resilient and susceptible cohorts in the present study and logistic regression showed no significant relationship between the proportion of Durusdinium pre-bleaching and post-bleaching mortality (*p=0.342*). These findings indicate that symbiont community composition was not a primary driver of mortality following complete bleaching (Fig. 1c, Fig. S2a, S2b)."

Main Methods Section: "A logistic regression analysis was performed to evaluate the relationship between the proportion of *Durusdinium* symbionts pre-bleaching and coral mortality post-bleaching (Dataset S1b). Further details found in SI Methods."

Supp. Methods Section: "A logistic regression analysis was conducted to assess the relationship between the proportion of *Durusdinium* symbionts pre-bleaching and coral mortality post-bleaching. Dataset S1b included coral genets classified as either "resilient" (survived) or "susceptible" (died) and their respective proportions of *Durusdinium* symbionts. Rows with missing data were excluded from the analysis. The binary outcome variable (mortality) was coded as 1 for "susceptible" and 0 for "resilient." The model was fitted using the "*glm"* function in

R with a binomial family to account for the binary nature of the response variable. Statistical significance of the relationship was assessed using *p*-values for the proportion of *Durusdinium* symbionts as the predictor variable."

L317-318: But in the results you state that this difference is not significant prior to bleaching, so this statement feels quite misleading. You could include the actual mean values of lipid content between groups in the results, and then clarify that the differences were 'trending' or something similar to this point in the discussion here. Regardless, the p-value and additional information needs to be included for the reader to interpret these results.

Author Response: Thanks for the suggestion, we have included p-values earlier in the text, and again here and modified the sentence:

Revised/new sentence: "No significant difference in total lipid content between T_1R and T_1S cohorts was found revealing that lipid content is not a predictor of survivability during this study (T_1R vs. T_1S: p=0.187; Fig. 2e)."

L411: Change 'advent' to 'onset'.

Author Response: done.-Thanks

L485: I don't think the word 'yolk' is appropriate to describe coral gametes, I suggest removing the word entirely and the sentence will make more sense.

Author Response: We have replaced the word yolk with lipid. The modified sentence now reads,

Revised/new sentence: "This could be a biomarker of decreased lipid provisioning to developing gametes in bleached resilient corals since this species only transfers autotrophic carbon to its gametes (Padilla-Gamiño et al., 2012a; Rodrigues & Padilla-Gamiño 2022; Jaffe et al., 2023)."

L487-488: Formatting of your references in this sentence is not aligned with the rest of the manuscript.

Author Response: Sorry and thanks for catching this- fixed now.

L558: In this coral management and restoration implications section, you elaborate in great

detail on how a biomarker assay would work for proteins, but don't mention details of the microbiome biomarkers at all. I am not convinced that quantifying diversity of the microbiome would serve as a biomarker, and instead you would need to have directly linked particular microbial taxa to bleaching tolerance. Please remove the microbiome biomarker discussion here, and focus instead on the proteins as you do have more clear evidence for those proteins.

Author Response: Thank you for the suggestion, we have clarified the section in response to the reviewers comment:

Revised/new sentence: "We identified two key biological factors that distinguish resilient from susceptible corals before the onset of thermal bleaching that forecast their long-term health: protein abundances (Fig. 2a,c) and microbiome diversity (Fig. 4a). Here, we focus on the proteomic results as we have no conclusive evidence that links a particular microbial taxa to bleaching tolerance, although recent research suggests that the microbiome may influence thermotolerance (Baquiran et al., 2024). As a high-throughput technology, proteomic biomarkers can be tailored to provide informative results on the molecular-level health of *M. capitata* across a range of resolutions…..."

Baquiran, Jake Ivan P., John Bennedick Quijano, Madeleine JH van Oppen, Patrick C. Cabaitan, Peter L. Harrison, and Cecilia Conaco. "Microbiome stability is linked to coral thermotolerance." bioRxiv (2024): 2024-10.

L586: change 'analyses' to 'sequencing'

Author Response: done

Methods

L602: Please include the depth range that corals were collected from, as the context of depth and therefore light environment is important to contextualize thermal tolerance.

Author Response: Thank you for insightful comments- The details have now been added

Revised/new sentence:

"Coral Collection and Experiment. Seventy-four *Montipora capitata* (approximately 30 cm in diameter) were collected from at 1.5-2 m depth Moku O Lo'e island (patch reef) located in Kāne'ohe Bay, O'ahu, Hawai'i (21.428°N, 157.792°W) in August 2017."

L602-626: Please indicate the light conditions that these corals were kept under for acclimation and for the experiment. Did anything change between acclimation and experiment? How do these light levels compare to what the corals experience on the reef?

Author Response: Thank you for your suggestion. We have included the following information, in addition to new Fig S1b:

" Mean daytime photosynthetic active radiation (P A R) levels in the tank s were 58 4 µ mol photons m −2 s−1, and mean P A R at 12: 00 was 1, 24 9 µ mol photons m −2 s−1 , measured using a waterproof P A R logger (Odyssey , D ataflow S ystems Limited, N Z ; Fig. S 1b). These irradiance values were very similar to values in the field where the coral colonies were located (Padilla-Gamiño et al. 2013). For more information on the experimental setup please see Timmins Schiffman 2024 and Axworthy et al. 2022."

L602-626: While you have included the target temperatures for the treatments, I don't think it is appropriate to point the reader to the supplemental data file to look for the actual temperature. Please either include a supplemental figure that plots temperature over time or summarize the actual treatment temperatures in this paragraph.

Author Response: Thank you and we apologize for the incomplete methods section and difficulty finding the data. We have included detailed information about the temperature in the text now and have included a a new plot of temperature (Fig. S1a).

Revised/new sentence:

"**Coral Collection and Experiment.** Seventy-four *Montipora capitata* (approximately 30 cm in diameter) were collected from at 1.5-2 m depth Moku O Lo'e island (patch reef) located in Kāne'ohe Bay, O'ahu, Hawai'i (21.428°N, 157.792°W) in August 2017. Corals were brought to shore and acclimated in flow-through outdoor tanks at the Hawaii Institute of Marine Biology (HIMB) for two weeks at ambient conditions (27± 0.4 °C, max=28.1, min=25.7). At the time of collection, corals were divided into two pieces to compare physiological performance for the same genets with/without exposure to thermal stress (Fig. 1 a, b). In September, one half of each coral was exposed to warmer water temperatures to simulate a natural thermal bleaching event[53]. To reach the 30°C temperature target for the bleaching treatment, experimental tank temperatures were increased by ~ 0.5°C per day for four days using a 600 W Titanium Aquarium Heater (Bulk Reef Supply, United States). Throughout the bleaching experiment, the control tanks had a temperature of 28 ± 0.6°C (range: 26.5-30.7°C), and the high-temperature tanks had a temperature of 30 ± 0.5°C (range: 28.9–31°C)(Fig. S1a)."

L611-612: Please include how many replicate tanks you had per treatment.

Author Response: Corals were placed in one of three replicate tanks per temperature treatment and rotated among tanks within a treatment each week to minimize tank effects.

Revised/new sentence: "Throughout the bleaching experiment, the control tanks had a temperature of 28 ± 0.6°C (range: 26.5-30.7°C), and the high-temperature tanks had a temperature of 30 ± 0.5°C (range: 28.9–31°C)(Fig. S1a).

Corals were rotated once a week among the same treatment tanks to minimize tank effects."

L619: What depth were these tables placed at, and does it match the depth range the corals were collected from? Please add more specific details here.

Author Response: Coral collections and racks locations occurred at a similar depth (1.5-2 m). Temperature and light conditions in the tanks matched conditions in the field.

This text has been included. "After thermal exposure, all corals (bleached and not bleached) were placed on racks at 1.5 - 2 m depth off HIMB (where they were originally collected from) to monitor survival and physiological recovery in situ for eight months."

L640: Please add the detail of what wavelengths you measured absorbance at.
Author Response: We have added those details:

Revised/new sentence: "Briefly, chlorophyll *a* was extracted with 100% acetone, and its absorbance was measured using a light spectrophotometer set to 663 nm (Table S1)."

L640: "triplicate ground coral tissue" is confusing here – do you mean you conducted triplicate counts from one slurry of ground coral tissue? Or did you grind three different portions of the coral sample and conduct individual counts from those? Please clarify.

Author Response: Yes, we conducted triplicate counts on each pellet.

We have modified the text accordingly,

Revised/new sentence: "Triplicate symbiont samples were taken from a single sample of ground coral tissue and homogenized prior to being counted using a hemocytometer."

L653-657: Please add information about the proportion that defined a community to be Durusdinium-dominated or Cladocopium-dominated, was it just >50%? And similarly, what defined a "mixed-community"? Also, we have a lot of evidence that particular species of Symbiodiniaceae really matter, are you able to distinguish the actual species of symbiont here or is this combining multiple species of each genera? If these characterizations include multiple species within each genera, I suggest changing the wording to be Durusdinium/Cladocopium

spp.

Author Response: We are not able to distinguish the species present - thus we have added spp. we have clarified the text so that the readers understand how the terms were applied.

We have modified the text to read “Symbiont community composition was classified as *Durusdinium*-dominated or *Cladocopium*-dominated if only one clade was present, or as a mixed community if both *Cladocopium* and *Durusdinium spp.* were present in any proportion, based on the symbiont community composition in samples collected at T_1 or from the non-bleached controls at T_2.”

L659-663: Please include details on the statistical analyses that you used to look for significant differences in lipids across your groups. It is unclear to me where to find this information.

Author Response: We used a two-way ANOVA to determine the single effects of bleaching resilience and bleaching status (from T1 to T2), as well as the interactive effects (status*resilience).

L682-683: Please indicate where you obtained this reference transcriptome (i.e., GenBank accession information).
Author Response: In addition to the reference with all the detailed links, we have added the following details :

Revised/new sentence: “Data were searched against a translated *M. capitata* transcriptome from NCBI Transcriptome Shotgun Assembly (TSA) database accession GFRO01000000[107] (GSE97888_Montiporacapitata_transcriptome.fasta). “

L701-725: You are missing a lot of details necessary to understand your microbiome analyses, and some of the details are not consistent between the main text and the supplement. Overall, my suggestion is to replace what you have in the main text with your supplement, as that contains all of the information I am looking for. But, here are a few examples of some issues I have with this section:
• You mention Mr. DNA for library prep and sequencing in the main text, but Molecular Research LP in the supplement. Please confirm what was actually done for sequencing.

Author Response: Thank you for your suggestion - this was difficult to write initially as there is a word count limitation and we had several methods used in the study. As you suggested, we have now moved the microbiome analyses into the main text and clarified the sampling, preparations, and statistical analysis used.

Now in main text:

Microbiome 16S rRNA Sequencing. Total DNA was extracted from the same subset of flash frozen coral fragments selected for the proteomic study (n=12) using the Qiagen DNA extraction kit. Sample purity was measured using a NanoDrop ND1000 spectrophotometer (NanoDrop Technologies, Willmington DE). Samples showing good quality and concentrations were sent to Molecular Research LP (Shallowater, TX) for library prep and 16S rRNA amplicon sequencing. Library prep and sequencing was performed following details outlined in Brown et al.[109] at Mr. DNA, Molecular Research LP. Briefly, 16S rRNA amplicons were amplified using Caporaso et al.[110] 515f (5′-GTGCCAGCMGCCGCGGTAA-3′) 806r (5′-GGACTACHVGGGTWTCTAAT-3′) primers. Amplification conditions were a single step 30-cycle PCR using HotStarTaq plus Master Mix (Qiagen) at the following temperatures: 94°C for 30s, 53°C for 40s, and 72 °C for 1 minute, followed by a final incubation step of 72 °C for 1 minute after all cycles finished. Sequencing was performed on an Ion Torrent PGM . All 16S rRNA gene amplicon sequence data, processing steps and code for quality control on the microbiome data and analysis are available on GitHub (https://github.com/tanyabrown9/Resilient_vs_Susceptible_Mcapitata). Sequences are deposited in NCBI as bioproject PRJNA933787. Initial sequencing resulted in a collection of 2,472,819 total reads, with an average read depth of 68,689 (± 28,107 SD) sequences per sample (Dataset S4a,b).

Microbiome 16S rRNA Data Preparation and Quality Control. 16S rRNA gene amplicon sequence data from Molecular Research LP were processed using the QIIME2 software package[20]. Forward and reverse reads from Molecular Research LP were imported into QIIME2 and demultiplexed (using the qiime paired-end sequencing method). Sequences were then denoised using DADA2 and chimaera-checked (using the qiime dada2 denoise-pyro method) to generate amplicon sequence variants (ASVs)[21]. Care was taken to identify mitochondria and chloroplasts *in silico* prior to downstream analysis as previously described[22]. Once annotated, chloroplast and mitochondria sequences were removed. Samples were rarefied to 10,000 sequences per sample to protect against false-positive results attributable to unequal sequencing depth between samples[25].

Microbiome alpha and beta diversity analysis. Alpha and beta diversity were assessed on rarefied samples after the quality control steps. To conduct phylogenetic alpha and beta diversity metrics, a fragment insertion approach was used to insert short microbial 16S rRNA reads into a reference phylogeny. Sequences were placed on the QIIME2-compatible SILVA 128 tree included with the SEPP software using the QIIME fragment-insertion SEPP method[23,24].

Alpha diversity was assessed samples rarefied to 1000 sequences per sample, using mutliple metrics: the number of unique observed ASVs in rarefied samples (observed features), Faith's Phylogenetic Diversity, Simpson's Evenness, and Shannon's Diversity Indexes. Overall differences in alpha diversity across susceptibility status and time points were tested using Kruskal-Wallis tests. False Discovery Rate (FDR) for pairwise tests between susceptibility and time was controlled using FDR q-values reported by QIIME2. Beta diversity between samples was assessed using Weighted UniFrac distances and Bray-Curtis dissimilarities. The

significance of differences in beta-diversity between susceptibility vs. resilient corals and across time points time was tested using PERMANOVA[112].

Microbiome Differential Abundance Analysis. The top 10 bacterial families in each sample type were selected for taxonomic analysis. Multivariate Association with Linear Models were performed on the 16S datasets using the R package MaAsLin2[50]. Fixed effects for the analysis included bleaching status, resilience, time point, and whether the colony survived or succumbed to thermal stress (bleaching) by T_2. Coral colony term was set as a random effect and the analysis method was “LM”. Results were considered significant if the q-value was <0.05.

Microbial Sequence Data Quality Analysis and Processing.
All other statistical analyses of microbial taxonomy were conducted using R 4.0.3. The top 10 bacterial families in each sample type were selected for taxonomic analysis. Significant differences between bacterial families, susceptibility, and timepoint were carried out using a nested ANOVA. The Benjamini-Hochberg FDR was used to control the false discovery rate. Microbiome Multivariate Association with Linear Models was performed on the 16S data using the R package MaAsLin2[27]. Fixed effects for the analysis included bleaching status, resilience, time point, and whether the colony survived or succumbed to thermal stress (bleaching) by T_2. Coral colony term was set as a random effect and the analysis method was “LM”. Results were considered significant if the q-value was <0.05.

Microbiome Functional Prediction Analysis. Predicted functional profiles for MetaCyc categories were generated from 16S rRNA data using PICRUSt2(Douglas, Maffei et al. 2020) . This method estimates gene content or other functional parameters based on phylogenetic modeling of 16S rRNA sequences relative to sequenced reference genomes. SETé enabled phylogenetic placement (SEPP(Mirarab, Nguyen et al. 2012)) was used for phylogenetic placement (--p-placement_tool sepp), while phylogenetic independent contrasts (PIC) was used for phylogenetic inference (--p-hsp-method pic). Predicted profiles of MetaCyc functional annotations at T1 were compared with ANCOM-BC(Lin and Peddada 2020) to identify differentially abundant functional categories (Fig. S6, Dataset S5).

L714: QIIME 2 and DADA2 are separate software, but QIIME 2 integrates DADA2 through a plugin (see info here: https://docs.qiime2.org/jupyterbooks/cancer-microbiome-intervention-tutorial/020-tutorial-upstream/040-denoising.html). Please update this sentence to be more clear. I strongly recommend including details on rarefication in the main text, as you only have it in the supplement.
Author Response: See new sections written for the microbiome analyses (addresed above). We have clarified all points as requested and included details on rarefication in the main text.

Figures

Figure 1:

Please clarify why the sample size changes from n=6 to n=4 for symbiont community composition of susceptible corals. In general throughout the manuscript, I got confused with genet- and ramet-level replication. Please add clarification of the sample size of individual genotypes and the number of fragments per genotype throughout the manuscript as appropriate.

Author Response:

Since symbiont community composition was determined at a later date than symbiont density, tissue samples for DNA extraction and symbiont identification via qPCR were only available for 4 susceptible coral genotypes. In addition to the sentence added below, we have gone through figure captions and where a sample number is mentioned (i.e., n=6) we clarify that these are genets.

Added to figure caption:

"The variation in the number of genets analyzed was due to limitations in the size of the archived sample collection."

Figure 2:

L788: ('grey boxes "B" boxes') is unclear as written. I'd recommend changing 'boxes' to 'bars' in this legend since it is a barplot. Also, I think you could just say something like 'grey bars with "B" labels' and that would be more clear.

Author Response: done- thanks for the suggestion.

Figure 3:

L792: Please define NSAF here.

Author Response: I have included the definition of NSAF in the text.

Revised/new sentence: "NSAF (Normalized Spectral Abundance Factor) is a quantitative metric used in proteomics to estimate the relative abundance of proteins in a sample based on spectral counting. It accounts for variations in protein length and the total number of spectra in a dataset, enabling a more accurate comparison of protein abundance across samples. NSAF normalizes the spectral count of a protein by dividing it by its length, then dividing this ratio by the sum of all spectral count-to-length ratios for all identified proteins in the sample."

SC_i: Spectral count for protein iii

L_i: Length of protein iii

N: Total number of proteins in the dataset

$$\mathrm{NSAF}_i = \frac{\frac{\mathrm{SC}_i}{\mathrm{L}_i}}{\sum_{j=1}^{N} \frac{\mathrm{SC}_j}{\mathrm{L}_j}}$$

Figure 4:
Please add a label to the legend that indicates the significance of the size of each dot. It seems to me like you could just say “log-transformed counts” and then include the same details you have about those counts being phylum-level in the legend.
Author Response: Thank you. This has been added to the figure legend and in the figure there is a clearer description of the dot size.

Revised/new sentence: “Size of the dot represents the log transformation of the phylum-level counts as indicated in legend presented in panel a.”

Figure 5:
Similar to Figure 3, please identify NSAF here.

Author Response: I have included this again at Fig. 5 legend and there is a detailed description of NSAF in the Supplemental information/ method section.

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