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Peer Review File



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Web links to the author's journal account have been redacted from the decision letters as indicated to maintain confidentiality.

Dear Dr Beinart,

I hope you are well?

Your manuscript titled "Deep-sea Pompeii: Hydrothermal vent communities buried by Hunga Tonga-Hunga Ha'apai volcanic ash" has now been seen by 3 reviewers, and I 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.

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. In addition to the comments provided by the reviewers, please also consider the following editorial thresholds as you revise your manuscript:

- Provide compelling evidence for oxygen availability as a primary driver for mass mortality in hydrothermal communities following ash sedimentation, including contextual discussion of other potentially viable mechanisms.

- Provide quantitative observations to support your interpretations wherever possible.

- Present a clear and logical narrative, which may require some restructuring of the text.

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) and the completed checklist:

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** 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.

We understand that due to the current global situation, the time required for revision may be longer than usual. We would appreciate it if you could keep us informed about an estimated timescale for

resubmission, to facilitate our planning. Of course, if you are unable to estimate, we are happy to accommodate necessary extensions nevertheless.

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

Best regards,

Emma Nicholson, PhD Editorial Board Member Communications Earth & Environment orcid.org/0000-0003-1749-9285

Joe Aslin Senior Editor Communications Earth & Environment

EDITORIAL POLICIES AND FORMATTING

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- Unique identifiers (such as DOIs and hyperlinks for datasets in public repositories)
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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 <u>http://www.nature.com/sdata/policies/repositories</u>.

If a community resource is unavailable, data can be submitted to generalist repositories such as <u>figshare</u> or <u>Dryad Digital Repository</u>. 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.

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REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

This is a fascinating, well-written manuscript, which we enjoyed reading. These sites provide an exciting opportunity to examine how hydrothermal communities respond to substantial ash sedimentation, and highlight the selectivity of this event on species mortality. At present, the manuscript is primarily descriptive; quantitative biological data, such as population counts or areal extent metrics, would help to illustrate the scale of mortality and highlight the fundamental shifts in taxon abundance with statistical significance, but we appreciate that due to the remoteness of the sites, such information may not be available.

The argument for oxygen availability as a primary driver for mortality is intuitive, but other drivers, such as stress-induced chemosymbiont loss, may better explain the patterns of mortality observed. We have included a brief discussion of this alternative hypothesis, which is comparable to coral bleaching, where symbionts are lost due to protracted environmental stress to the host. This appears to be consistent with the preferential loss of chemosymbiotic organisms in the studied sites. We hope that consideration of this alternative hypothesis will be beneficial to the manuscript, at the very least providing a comparative model against which to test the idea that oxygen availability is the primary driver of mortality.

This manuscript will undoubtedly promote future studies into the recovery of communities following major volcanic eruptions, both in modern settings and in the geological record. We hope that the

comments below are helpful to the authors going forward, and we would be happy for you to contact us to discuss any of these points further.

1. Data, statistics, and sedimentation

This manuscript documents changes in vent community composition and structure arising from the Hunga Tonga Hunga Ha'apai volcanic eruption. While the authors provide a wealth of supplementary ROV videos and figured comparisons of sites before and after the eruption, it is left to the reader to assess the significance of the changes in faunal distributions. Quantitative data – either rough censuses or areal coverage – would help to illustrate any decreases in specific taxa, and would provide a means to test the statistical significance of the relative changes in faunal makeup whilst minimising subjectivity. The segregation or aggregation of taxa may even provide insight into the spatial patterns of mortality in the communities, relating to the distribution of sediment at the time of deposition. This could in turn permit distinction between smothering by gradual ash accumulation via slow ash fall, versus rapid burial via sedimentary flows, if such spatial data are feasible to derive from the ROV dataset.

As an aside, the presence of large anemones in some of the images (presumably too large to be colonising the area since the eruption?) is of interest. Do you consider them to have survived the event? If so, this could indicate gradual ash fall as a more likely mechanism of sediment delivery. Future searches for sedimentary structures within the deposits would provide a straightforward way to independently distinguish between possible sediment delivery mechanisms.

2. An alternative kill mechanism

The suggestion that the observed patterns of diversity loss can simply be explained by the combined effects of ash burial and taxon-specific oxygen limitations is intuitive, but there is an alternative explanation. The enhanced gills of chemosymbiont-bearing taxa are not a result of oxygen limitation for the host, but rather they are designed to enhance chemosymbiont metabolite uptake (Childress and Girguis, 2011; https://doi.org/10.1242/jeb.049023). During periods of environmental stress, chemosymbionts may be culled by the host to provide autotrophically-derived carbon to supplement the host's diet , or to prevent breakdown of the mutualistic host-chemosymbiont relationship (Reynolds and Rolff, 2008; https://doi.org/10.1186/jbiol88), or they may simply be lost due to decreasing gill size with host starvation (Elisabeth et al., 2014; https://doi.org/10.1111/1574-6941.12366). With dramatic reduction of chemosymbiont populations, the hosts would be more susceptible to pathogens (Détrée et al., 2019; https://doi.org/10.1186/s12864-019-5456-0), accumulate more toxic chemicals (Bojar et al., 2023; https://doi.org/10.1016/j.chemosphere.2023.138258), and be unable to maintain body weight (Elisabeth et al., 2014; https://doi.org/10.1111/1574-6941.12366). Effectively, this could feasibly result in an outcome comparable to coral bleaching, where the stress response of the host (loss of symbionts) invariably results in further stress and enhances mortality.

Of the three dominant taxa in this study, Alviniconcha exhibits the greatest dependency on chemoautotrophy – with metabolite uptake rates comparable to Riftia, corroborated by nitrogen isotopes (Henry et al., 2008; https://doi.org/10.1016/j.dsr.2008.02.001), and a highly atrophied – though

functional – stomach (Laming et al., 2020; https://doi.org/10.1186/s12983-020-00357-x). Bathymodiolus retains a gut and filter-feeding capabilities and supplements its predominantly autotrophic mode of life via heterotrophy (Dubilier et al., 1998; https://doi.org/10.3354/meps165187). Ifremeria exhibits the least dependence on autotrophy, with nitrogen isotopes indicating occupation of a higher trophic niche than Alviniconcha (Henry et al., 2008; https://doi.org/10.1016/j.dsr.2008.02.001). Nonetheless, when unstressed, Alviniconcha, Ifremeria, and Bathymodiolus are all primarily autotrophic. When stressed, Bathymodiolus gradually lose their chemosymbionts during limited sulfide availability (Kádár et al., 2005; https://doi.org/10.1016/j.jembe.2004.12.025), possibly via lysis (Piquet et al., 2022; https://doi.org/10.3389/fmars.2022.968331). Such behaviour is also observed in Codakia orbiculata, with rapid lysis of chemosymbionts upon depletion of sulfur granules within the gills (Elisabeth et al., 2014; https://doi.org/10.1111/1574-6941.12366). Chemosymbiont lysis could not sustain C. orbiculata, with significant gill tissue loss (~50%) within 6 months (Elisabeth et al., 2014; https://doi.org/10.1111/1574-6941.12366). During periods of stress, Alviniconcha also undergoes chemosymbiont loss, though via physical expulsion of mats from the pallial cavity (Sigwart and Chen, 2018; https://doi.org/10.1086/699326). The effect of stress on the chemosymbionts hosted by Ifremeria is not well-known, though evidence of cannibalism - and thus heterotrophy - in captive specimens (Henry et al., 2008; https://doi.org/10.1016/j.dsr.2008.02.001) hints at a similar departure from reliance on autotrophy during periods of stress. This is, however, purely conjecture. It seems from the data presented that Alviniconcha, with the greatest chemosymbiont dependence (Henry et al., 2008; https://doi.org/10.1016/j.dsr.2008.02.001), was amongst those most profoundly affected by the Hunga Tonga Hunga Ha'apai eruption at your studied sites.

The impacts of chemosymbiont loss are significant and wide-ranging; in Bathymodiolus, chemosymbiont loss results in a heightened immune response and enhanced apoptosis within gill cells (Détrée et al., 2019; https://doi.org/10.1186/s12864-019-5456-0), suggesting susceptibility to pathogens. Additionally, chemosymbionts provide an important organic carbon source for the host; though most chemosymbiont-bearing taxa retain a certain degree of heterotrophic capability (Dubilier et al., 1998; https://doi.org/10.3354/meps165187), they exhibit evidence of starvation without chemosymbionts (Elisabeth et al., 2014; https://doi.org/10.1111/1574-6941.12366). Chemosymbionts in Alviniconcha and Ifremeria (and likely Bathymodiolus) also catalyse the oxidation of reduced arsenic species, decreasing the toxicity of such compounds to the host (Bojar et al., 2023;

https://doi.org/10.1016/j.chemosphere.2023.138258). With chemosymbiont loss, the host organism will be exposed to a greater proportion of reduced arsenic species, dramatically increasing arsenic toxicity.

During periods of enhanced sediment flux, Bathymodiolus would likely exhibit valve closure (Nerlović et al., 2011; https://doi.org/10.2478/s11756-011-0121-3), generating a microenvironment within the valves of the shell. Alviniconcha and Ifremeria both possess opercula (Laming et al., 2020; https://doi.org/10.1186/s12983-020-00357-x and Bouchet and Waren, 1991; http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=19578750 respectively) capable of minimising sediment influx. Without modulation of chemosymbiont metabolic rates, generating such closed systems would result in rapid depletion of metabolites to the detriment of the host and its chemosymbionts (e.g. Raulfs et al., 2004; https://doi.org/10.1017/S0025315404009087h). During periods of intense nutrient stress, chemosymbionts may transition toward heterotrophy, favouring metabolism of the host's tissues (Piquet et al., 2022; https://doi.org/10.3389/fmars.2022.968331). Such

a breakdown of the chemosymbiont-host relationship would necessitate a significant immune response (Reynolds and Rolff, 2008; https://doi.org/10.1186/jbiol88) and apoptosis of bacteriocytes (Zheng et al., 2017; https://doi.org/10.1111/mec.14160) to mitigate damage to the host. Here, host-chemosymbiont integration is insufficient to permit host adjustment of chemosymbiont metabolic rates, suggesting that the sole control on chemosymbiont metabolite uptake is via host-mediated chemosymbiont lysis (Zheng et al., 2017; https://doi.org/10.1111/mec.14160). Given that hemipelagic ash settling was recorded for two months post-eruption (Wei-Haas, citation 13), protracted exposure to suspended ash was a near-certainty for all taxa at these sites. Simultaneously, decreased heterotrophic efficiency due to ash dilution of bioavailable particulate matter would increase the rate of chemosymbiont lysis within the bacteriocytes to mitigate shortfalls in energy availability. These processes would be expected to have a profound impact on chemosymbiont numbers, dramatically reducing the availability of autotrophically-derived carbon and energy to the host. This may be reflected by shifts in carbon isotope fractionation or observed by taking histological sections through gill tissues, and is therefore potentially testable in the future.

3. Minor comments

I. 99. Could you provide a little more detail of these ongoing analyses?

I. 109. Perhaps clarify whether these dilute turbidity currents are being invoked to explain the sedimentation at these sites.

I. 128. Perhaps discuss these mats further – do they represent the first stage of succession? Or just a short, opportunistic occupation?

I. 145-162. See section 2 of this review. Is it possible to test between these hypotheses?

I. 155. What is the taphonomy of these un-buried empty shells? Were they always exposed, or could currents have re-exposed them by re-suspending the ash?

I. 170. Could you elaborate on which aspects of vent fluid chemistry have shifted here? (i.e. are there any specific elements that are more or less important?)

I. 191-192. Or, reproduction of the few (<20) surviving taxa! If this is the case, early colonisation of Tow Cam by a very genetically-restricted group of descendants would be expected to yield increasing genetic diversity and gene flow through time.

Figure 1: Your figures and ROV videos are excellent! Just a passing thought – are the Globigerina-like foraminifera likely to have rained into the deposits post-eruption, or are they also victims of the eruption? Could the vertical density currents suggested to have entrained the ash also have entrained foraminifera?

Figure 2: It would be helpful to state the organisms comprising the microbial mat in b) in the caption. In d), are the anemones survivors of the event, or recolonisers? If the former, it demonstrates remarkable resilience of those specific organisms that might be worthy of note in the paper.

Figure 4: How much sediment do you think has been remobilised and resuspended/removed from the sites in the period between eruption and filming? Is that likely to have impacted the results of this study? I. 440: Perhaps consider including a statement on how much, if any, fine particulate material would have been lost during the sample scooping process.

Best wishes,

Reviewer #2 (Remarks to the Author):

This manuscript presents exciting new work on the effects of volcanic ash deposition on marine hydrothermal vent communities. The authors took advantage of an unprecedented opportunity to study the effects of ash deposition on marine communities following a large volcanic eruption, and the work should be of interest to a wide variety of workers in the fields of marine biology, ecology, geology, geochemistry, and paleontology. It is also exceptionally well-written, clearly organized, and sets a clear foundation for future work on the subject. Therefore, it is the opinion of this reviewer that the manuscript is appropriate for publication in Nature Communications Earth & Environmental Science.

The paper presents clear qualitative evidence that benthic marine communities can experience mass mortality as a result of a volcanic eruption on the order of 80-100 km from the provenance, and appears to show variable survivability of such an event. However, it would be greatly strengthened by the addition of a more quantitative analysis of the data. A quantitative analysis would make this study more readily comparable to previous and future studies, and strengthen the core claims of mass mortality and variable survivability of marine taxa following a large-scale volcanic eruption.

I have provided additional comments on a .pdf of the manuscript to be considered at the discretion of the author and editor, and will include some citations below which may be of interest to the authors:

Crawford, R.S., Casadío, S., Feldmann, R.M., Griffin, M., Parras, A., Schweitzer, C.E., 2008. Mass mortality of decapods within the Monte Leon Formation (Early Miocene), Southern Argentina: victims of Andean volcanism. Ann. Carnegie Museum 77, 259–287.

Maguire, E.P., Feldmann, R.M., Casadio, S., Schweitzer, C.E., 2016. Distal Volcanic Ash Deposition As a Cause for Mass Kills of Marine Invertebrates During the Miocene in Northern Patagonia , Argentina. Palaios 31, 577–591.

Maguire, E.P., 2022. The Effect of Volcanic Ash Deposition on Marine Environments, Invertebrate Ecosystems and Fossil Preservation (dissertation, chapters pending publication)

Feldmann, R.M., Franţescu, A., Franţescu, O.D., Adiël, A., Logan, G., Robins, C.M., Schweitzer, C.E., Waugh, D.A., ... , 2012. Formation of lobster-bearing concretions in the Late Cretaceous Bearpaw Shale, Montana, United States, in a complex geochemical environment. Palaios 27, 842–856

Palópolo, E.E., Kroh, A., Harzhauser, M., Griffin, M., Casadio, S., Carmona, N., 2021. An early Miocene spatangoid assemblage on a submarine volcanic ash dune from Patagonia (Argentina). J. South Am. Earth Sci. 108

Wall-Palmer, D., Jones, M.T., Hart, M.B., Fisher, J.K., Smart, C.W., Hembury, D.J., Palmer, M.R., Fones, G.R., 2011. Explosive volcanism as a cause for mass mortality of pteropods. Mar. Geol. 282, 231–239

Orr, P.J., Briggs, D.E.G., Siveter, D.J., Siveter, D.J., 2000. Three-dimensional preservation of a nonbiomineralized arthropod in concretions in Silurian volcaniclastic rocks from Herefordshire, England. J. Geol. Soc. London 157, 173–186.

Hyžný, M., Hudáčková, N., Szalma, Š., 2016. Taphonomy and diversity of Middle Miocene decapod crustaceans from the Novohrad-Nógrad Basin, Slovakia, with remarks on palaeobiography. Acta Geol. Slovaca 7, 139–154.

WIESNER, M.G., WANG, Y., ZHENG, L., 1995, Fallout of volcanic ash to the deep South China Sea induced by the 1991 eruption of Mount Pinatubo: Geology, v. 23, p. 885–888

CAREY, S., 1997, Influence of convective sedimentation on the formation of widespread tephra fall layers in the deep sea: Geology, v. 25, p. 839–842

Please feel free to contact me if there are any questions regarding this review.

Reviewer #3 (Remarks to the Author):

This work touches on the extremely interesting and little studied topic of the dynamics and recovery of hydrothermal communities after natural disturbance. As rightly noted the existing data on this topic are very scarce and concern only a few areas, while in other areas this issue has not been studied at all. Nevertheless, the issue of recovery of hydrothermal communities is very important, not only from a theoretical point of view, but also from a practical one, given the prospect of extracting mineral resources from such areas. The authors managed to record an unusual moment when the hydrothermal communities were covered with volcanic ash, which had not previously been recorded at all. Having data on the state of hydrothermal communities several years before the volcanic eruption, the authors were able to assess the catastrophic changes in communities that occurred after the eruption. These data can be a good starting point for further observations of the dynamics of hydrothermal communities after natural disturbance. The paper presents the very first data concerning the most general visual observations of megafauna. Despite this, these observations are of great value and I would very much like to wish the authors to continue their work and more detailed studies in the future.

I would like the manuscript to be more structured into sections. The present chapter Results and Discussion contains in part paragraphs that are more related to the Introduction or Methods. In turn, the Introduction chapter contains information that should be included in the Results. It would also be better to separate the results and their discussion into different sections. The results should be described in more detail involving quantitative estimates. The authors have written the Discussion well, summarizing and analyzing most of the existing work on the dynamics and recovery of hydrothermal biotopes. I recommend major revisions for this manuscript.

I'll give some specific comments below.

L. 76. Give a title to all subsection of a section «Results and Discussion».

L. 68-71. It is the main results and they shouldn't be in the Introduction section.

L. 77-80. This needs to be moved to the Introduction.

L. 80-83. It's more about methods.

L. 86-87. This would be better placed in the Introduction.

L. 97-111. This is not entirely relevant to the biological results obtained in the work. Some information from this paragraph can be inserted into the Introduction. It is also worth giving a subsection in Materials and methods - «Study area».

L.115. What species do you classify as macrofauna in your study? I think you are only describing megafauna.

L.115. Rather you mean not «density», but «frequency of occurrence».

L.131, L. 136. Could you give more numerical estimates for the frequency of occurrence of different groups of animals before and after the eruption at different vent fields?

L.133. Among the species remained around diffuse venting and on chimneys is of particular interest Vulcanolepas buckeridgeia. Firstly, I'm not sure if it can be attributed to «scavengers, predators, or grazers». They rather are filter-feeders. Moreover, it was noted that they are possibly ectosymbiotic with bacteria, farming the bacteria on the setae for food (Chan and Chang, 2018). What do you think may be the reason for the resistance of this sedentary species to the ash fall? The same question is about zoanthids? In the abstract you also need to insert filter-feeders.

L. 141: Before you wrote about «five active hydrothermal vent fields and one inactive field along the Eastern Lau Spreading Center-Valu Fa Ridge in the Lau back-arc basin». But now you describe only five fields. Why is there no information on the sixth field? What field is inactive? It is better to describe the fields in the subsection «Study area».

L. 145. Please, take a look at the article Nakajima et al., 2019 «Clams after storms: the impact of multiple disturbances on seep vesicomyid clams revealed by long-term monitoring». I think it should be included in the Discussion.

L. 145. Can ashes have any other negative effect besides oxygen deficiency and mechanical burying? For

example, a toxic effect, especially on suspension feeders and filter-feeders?

L.164. The title of the subsection should be reformulated.

L.167. «chemosynthetic biological communities» throughout the text it is better to replace with «chemosynthesis-based communities».

Supplementary Video 2. What objects are visible in the upper left corner and in the center?

Deep-sea Pompeii: Hydrothermal vent communities buried by Hunga Tonga-Hunga Ha'apai volcanic ash

4 R.A. Beinart^{1*}, M. Chaknova^{2,3,4}, S.M. Arellano^{5,6,7} D. Davis⁵, V. Jimenez^{5,6}, A. Calhoun^{2,3}, T.

5 Beaver^{5,6}, J. Becker⁸, S. Bergen^{2,3}, M. Betters⁹, L. Brunner¹⁰, E.J. Cowell⁹, M. Hauer¹, M.

Heffernan⁷, T.M. Ladd⁵, C.Q. Plowman^{2,3}, L.N. Rice^{2,3}, A. Taradash^{2,3}, T. Giachetti⁴, C.M.
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21 * Correspondence to <u>rbeinart@uri.edu</u>

47 Abstract

48	Mass mortality of marine animals due to volcanic ash deposition is common in the fossil record
49	but has rarely been documented in real time. Here, we describe the devastating effect of ash from
50	the record-breaking Hunga Tonga – Hunga Ha'apai 2022 volcanic eruption on endangered and
51	vulnerable snail and mussel species that previously thrived at nearby deep-sea hydrothermal
52	vents. In contrast to grazing, scavenging, and predatory vent taxa, we observed mass mortality of
53	the foundation species, which rely on symbiotic chemosynthetic bacteria for the bulk of their
54	nutrition. This is significant for our broad understanding of the natural disturbance of marine
55	ecosystems by volcanic eruptions and for predicting the effects of anthropogenic disturbance,
56	like deep-sea mining, on these unique seafloor habitats.
57	
58	Introduction
59	Rapid deposition of volcanic tephra following an eruption can cause mass mortality of
60	animal communities ¹ , though, in the ocean, this has been observed only rarely, even in shallow
61	habitats ^{2–5} . Fossilized aggregations of marine animals in volcaniclastic sediment and ash are
62	exceptionally well-preserved, providing historical evidence for the significance of these events
63	and subsequent shifts in faunal community composition ⁶ . However, the paucity of modern
64	observations of the effects of ash fall on marine communities means that we do not have the
65	depth of understanding regarding ecosystem or organismal response, resilience, and succession
66	after volcanic eruptions that we have for terrestrial ecosystems ¹ . Here, we report the first
67	observations of ash deposition from the Hunga Tonga-Hunga Ha'apai (HTHH) submarine
68	volcano (Kingdom of Tonga) at nearby deep-sea hydrothermal vents. At the sites most heavily
60	

and mussel (IUCN-designated endangered or vulnerable species) that dominated the seafloor of these habitats, leaving behind mainly motile crustacean scavengers and predators. This discovery provides the unprecedented opportunity to understand the impact of volcanic activity on deep-sea marine ecosystems, historically and in modern times, and to study community recovery and succession following a major volcanic event of unprecedented magnitude.

75

76 Results and Discussion

77 Eruptive activity at the HTHH volcano began on December 20, 2021, ending with a 78 record-breaking explosive eruption that sent a plume of material as high as 58 km on January 15, 79 2022^{7,8}. Up to 10 km³ of the seafloor was displaced from the caldera walls and flanks during this 80 eruptive period⁹. Approximately three months later (April 2022), we conducted a series of 81 remotely operated vehicle (ROV) dives at five active hydrothermal vent fields and one inactive 82 field along the Eastern Lau Spreading Center-Valu Fa Ridge in the Lau back-arc basin. These 83 ranged in distance from 83 to 222 km west of the HTHH caldera (Fig.1, Table S1). These dives 84 revealed a north to south gradient in ash sedimentation thickness, with the thickest deposits in the 85 northern vent fields and no apparent deposits at the most southern vent fields (Fig.1, Table S2). 86 These vent fields have been observed many times, most recently in 2019 during the CHUBACARC expedition¹⁰ and were not previously sedimented. We recovered over 25 kg of 87 88 sediment material by scoop from deposits ranging in thickness from 7-150 cm (Table S2, Table 89 S3). The thickest deposits were from Tow Cam (80-150 cm) (Fig.1, Table S2), the vent field 90 nearest to HTHH. The material collected was extremely fine-grained volcanic ash (89–99 wt.% 91 <63 µm) (Fig.1, Fig.S1,S2,S3). Grain size distribution was consistent at all vent fields with a 92 mean particle diameter of 26-31 µm (Fig.1, Fig.S3). The deposits were rich in juvenile volcanic

3

93	glass (>80% of point-counted grains) ranging from dense to pumiceous and contained 1–30%
<mark>94</mark>	lithics (Fig.1, Fig.S3). We interpret this material to have come from the January 2022 eruption of
95	HTHH based on the deposit thickness, grain size distribution, and the extreme freshness of the
96	juvenile glass (Fig.1, Fig.S1-3).
97	The mode of ash deposition at these sites is still under question. Two hypotheses have
98	emerged: subaerial fallout from the volcanic plume or submarine flow and consequential
99	resuspension of fine particles (<2 mm). Ongoing analyses suggest a combination of fall and flow
100	at these sites. The initial ash-containing volcanic cloud centered on HTHH had a maximum
101	diameter of 260 km ¹¹ , encompassing the visited vent sites (Fig.1). Ash fall was recorded for 10
102	hours on the island of Tongatapu, Kingdom of Tonga, 65 kilometers southeast of HTHH caldera
103	after the main eruption ¹¹ . Given the particle size distribution observed to the west (89–99 wt.%
104	<63um) (Fig.1, Fig.S3), sinking rates would have allowed ash to travel from the ocean's surface
105	to these seafloor vent fields (\sim 1,800 – 2,800 meters below the sea surface) within a few days to
106	weeks by rapid vertical settling currents ¹² . Settling of ash was observed in the water column two
107	months following the eruption ¹³ . The HTHH eruption also produced massive pyroclastic density
108	currents (PDCs) along the northwest and southeast flanks. It is suspected that the PDCs
109	transitioned into dilute submarine flows rapidly burying the seafloor with fine-grained ash. This
110	hypothesis is supported by the severance and burial of two seafloor fiber-optic
111	telecommunications cables to the south and southeast by ~ 30 meters of ash ⁹ .
112	
113	The impact of the HTHH eruption on Lau Basin hydrothermal vent communities
114	Regardless of its mode of arrival, the ash sedimentation observed here caused significant
115	changes in benthic mega- and macro-faunal density and community composition at three active

116 vent fields previously known to harbor abundant hydrothermal-vent associated animal

117 communities^{14,15}. Prior to the eruption, four of the vent fields we visited, Tow Cam, Tahi Moana,

ABE, and Tu'i Malila, were dominated by large populations of chemosymbiotic molluscs (snails

119 Alviniconcha boucheti, Alviniconcha kojimai, Alviniconcha strummeri, and Ifremeria nautilei,

120 and mussel *Bathymodiolus septemdierum*), that obtain their primary nutrition from bacterial

symbionts hosted in their gills^{16–18}. These vent fields were also previously inhabited by

122 heterotrophic grazers, filter-feeders, scavengers, and predators such as stalked barnacles, squat

123 lobsters, sea anemones, crabs, shrimp, and eelpout fishes^{16,17,19–22}. After the eruption, the active

124 vent field with the greatest ash deposition, Tow Cam, was almost completely devoid of the

125 chemosymbiotic animals, with only <20 individuals observed alive during our almost 30 hours

126 of dive time covering all previously known areas of venting. Instead, there were mainly large

127 areas of empty snail and mussel shells (Fig.2b-d; Supplementary Videos 1-3). At this site, we

128 observed ash-covered low-temperature diffuse venting areas with obvious white microbial mat,

129 likely sulfur-oxidizing bacteria (Fig. 2b,e,f; Supplementary Videos 1-3), as well as vigorously

130 flowing high-temperature hydrothermal chimneys (Supplementary Video 4). The conspicuous

131 mega- and macro-fauna that remained around diffuse venting and on chimneys were

132 hydrothermal-vent associated scavengers, predators, and grazers: *Austinogrea* spp. crabs,

133 *Munidopsis* spp. squat lobsters, *Rimicaris* spp. and *Alvinocaris* spp. shrimp, *Vulcanolepas*

134 *buckeridgeia* stalked barnacles, *Enigmaticolus desbruyeresi* whelks, and unidentified zoanthids.

135 Though not decimated like those at Tow Cam, the chemosymbiotic benthic animal communities

- 136 were also significantly impacted at the Tahi Moana and ABE vent fields, which were covered by
- 137 up to 15 cm of ash. At both fields, we observed small patches of living *I. nautilei* snails and *B.*
- 138 septemdierum mussels on chimneys and around diffuse flows (Fig.3a; Supplementary Video 5),

and even smaller numbers of large *Alviniconcha* spp. snails on chimneys at ABE only (Fig.3b;
Supplementary Video 6). As at Tow Cam, we observed abundant scavengers, predators, filterfeeders, and grazers. Tu'i Malila and Mariner, the vent fields farthest from HTHH, did not have
detectable ash deposition and had biological communities that were qualitatively similar to preeruption communities (Fig.4).

144

145 Rapid sedimentation likely caused mass mortality due to oxygen deficiency

146 Rapid sedimentation events are known to cause significant changes in benthic animal 147 abundance and taxonomic composition due to differential survival in suspended sediment or variable escape from burial^{23,24}. For mobile epibenthic organisms, survival after burial depends 148 149 on vertical migration to the sediment surface, which is a function of sediment depth and animal 150 motility $^{25-30}$. Epibenthic bivalves and gastropods, like the chemosymbiotic mussels and snails 151 here, are known to have varying responses to burial, but in general, have limited escape potential, especially in deep and dense sediments^{26,31–33}. Without escape, mortality increases with 152 153 sediment thickness and duration of burial, temperature, and with increasingly finer-grained 154 sediments, suggesting that oxygen deficiency is the ultimate cause of death, since these factors influence access to oxygen or respiratory rates^{31,32}. The unburied patches of empty shells at the 155 156 bases of chimneys and in some diffuse flow areas suggest that some chemosymbiotic snails and 157 mussels avoided burial but still experienced substantial stress, and ultimately mortality, during 158 this sedimentation event. Significant respiratory effects and an associated decline in health 159 condition have also been documented in shallow-water mussels subjected to suspended ash particles in the water column³⁴. Vent invertebrates hosting chemosynthetic symbionts have a 160

161 very high oxygen demand³⁵. Thus, they may be especially vulnerable to oxygen deficiency

162 during ash burial or even when exposed to suspended ash particles.

163

164 **Recovery of hydrothermal vent communities after ash deposition is unknown**

165 Mass mortality of hydrothermal vent animals due to the underwater expulsion of volcanic lava has been observed occasionally^{36–38} at areas of frequent tectonic and volcanic activity along 166 167 active plate margins and seamounts. The dense chemosynthetic biological communities typical 168 of these ecosystems are thought to experience recurrent natural disturbances varying in 169 magnitude from total eradication caused by chemical and physical effects of submarine eruptions³⁹ to milder perturbations caused by shifts in vent fluid chemistry^{40,41}. However, 170 171 previous observation of the natural disturbances experienced by deep-sea hydrothermal vent 172 communities has been exclusively limited to effusive seafloor eruptions that catastrophically paved over these habitats with solidified lava at vents along the Eastern Pacific Rise³⁷ and Juan 173 de Fuca Ridge^{38,42}. In these settings, the return to a near pre-eruption state occurred within only 174 175 about eight years through recolonization by planktonic larvae coming from both near and far sites^{39,43–46}. Similar studies of community recovery on lava-covered volcanic flanks in shallow 176 water suggest much longer recovery times in arctic ecosystems⁴⁷ and both slow and rapid 177 succession in tropical reef communities^{5,48–50}. Our understanding of the response of vent 178 179 communities to natural disturbance is biased by a limitation of prior observations to fastspreading ridges with a fast, decadal tempo of disturbances^{36,38}. However, results from fast-180 181 spreading ridges cannot necessarily be extrapolated to other volcanic systems. For example, 182 hydrothermal vents in back-arc basins, like those observed here, are thought to experience a 183 much slower pace of natural disturbance and have shown remarkable ecological stability at the

decadal scale^{19,51}, though models incorporating larval dispersal and population dynamics have
predicted that vent communities in the Lau Basin could recover from a disturbance in under five
years⁵².

187 In a sedimentation disturbance event, community recovery could potentially occur 188 through vertical migration through sediments after burial, lateral migration of adults or juveniles 189 from nearby habitats, or recolonization through larval dispersal and settlement. Given that the 190 vent fields are separated by distances too far for lateral migration by adults (9 - 212 km), 191 recolonization via larval supply from distant vents is likely the only pathway for recovery for the 192 decimated communities at Tow Cam. At the other vent fields, the remnant populations that persisted are likely to also be important for recovery⁵³. However, the significant change in 193 194 substratum type, from exposed basaltic and andesitic to a heavily sedimented seafloor, may inhibit or prevent recolonization by these hard-bottom species^{14,19} even when larvae arrive from 195 196 the local or regional pool.

197 Further observations of the vent fields impacted by the HTHH eruption have the potential 198 to expand our knowledge of natural disturbance in vent ecosystems, and of the mechanisms by 199 which such systems recover. The ash deposition we observed is a very different kind of 200 disturbance than the magmatic deposition events where succession has been studied elsewhere. 201 Moreover, the linear gradient in ash disturbance intensity along this back-arc basin offers an 202 unparalleled opportunity to follow recovery of vent communities that have been differentially 203 impacted by a single disturbance event. Such observations will yield important insights on the 204 resiliency of deep-sea chemosynthetic ecosystems in general, including those impacted by sedimentation associated with deep-sea mineral extraction.⁴⁸ 205

206

207 Main References

8

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Fig.1: a) Componentry (colored vertical bars) and grain size distribution (red line) of ash collected from Tow Cam; b) Scanning electron micrograph of bulk ash sample from Tow Cam. Particle color corresponds to examples of the particle categories selected for in componentry; c) Plume imagery at 4:46 UTC on January 15, 2022 provided by Himawari from the Data Integration and Analysis System (DIAS) by Japan Agency for Marine-Earth Science and

- Technology (JAMSTEC). Predicted submarine flow routes based on ash deposit thickness, and particle properties; d) bathymetry plot of sample sites, their corresponding distance from HTHH
- and ash deposit thickness. Vent field abbreviations are as follows: KM, Kilo Moana; TC, Tow
- Cam; TM, Tahi Moana; TuM, Tu'i Malila; Ma, Mariner.



Fig.2: ROV photographs from Tow Cam, the vent field with the greatest ash thickness (80-150 cm). a) thick ash deposits; b) thick ash deposits with patches of empty shells and white microbial mat, a new marker deployed on TN401 is also visible; c,d) empty shells of dead chemosymbiotic

snails and mussels among living crustaceans, anemones, and other grazers, scavengers, and filter feeders; e,f) hydrothermal chimneys covered in white microbial mats and surrounded by ash

- deposits.



- **Fig.3**: a) Patches of living chemosymbiotic *I. nautilei* snails and *B. septemdierum* mussels among
- 408 the ash deposits at the Tahi Moana vent field and b) *Alviniconcha* spp. and *I. nautilei* snails on a
- 409 hydrothermal vent chimney at the ABE vent field. Inset boxes highlight representative patches of
- 410 Alviniconcha spp. (Alv.) and I. nautilei (I.n.) snails.



- Fig.4: Comparison of 2019 pre-eruption conditions (left panels) and 2022 post-eruption
- conditions (right panels) at specific seafloor locations at active vent fields with maximal ash
- deposition, Tow Cam (a-d), and negligible ash deposition, Tu'I Malila (e,f). Comparative
- photographs of a larval collection device that was deployed in 2019 and then located again in
- 2022 (a,b) and a navigational marker (yellow arrows) (c,d) demonstrate the thick ash deposition
- at Tow Cam. Panels e and f show navigational markers at Tu'i Malila (orange arrows), a
- southern site with little detectable ash, among qualitatively similar communities of
- chemosymbiotic animals in both years, indicating little change in the communities since the eruption.

429 Methods

430 Thirteen dives with remotely operated vehicle (ROV) Jason II (National Deep 431 Submergence Facility, Woods Hole Oceanographic Institution) were conducted April 3-27, 2022 432 during cruise TN401 aboard the R/V Thomas G. Thompson (University of Washington). Five 433 active and one inactive hydrothermal vent fields, at depths ranging from ~ 1800 to ~ 2800 meters, 434 along the Eastern Lau Spreading Center-Valu Fa ridge were each visited on 1-3 separate dives. 435 Total dive time at each vent field, including 1-1.5 hour ascent and descent times, ranged from 436 ~14 to ~52 hours (Table S1). Ash thickness was measured by using a 61-cm metal probe marked 437 in 7.6 cm increments along its length that was held by the ROV manipulator arm and pushed into 438 the sediment until it hit seafloor rock below (Supplementary Video 7). Observations of animal 439 communities from video footage were qualitatively compared to the most recent previous work 440 in this area, the CHUBACARC 2019 expedition¹. Ash was collected by scooping with canvas 441 bags. 442 443 Componentry and grain size analysis of collected ash 444 Bagged ash samples consisted of 25 kg collected from 7 locations (Table S3). Particle 445 size distribution was carried out by wet and dry sieving. Bulk representative 5 g splits from each 446 location were wet sieved in half phi intervals down to 63 µm. A dilute concentration of Calgon 447 (Na₆O₁₈P₆) and DI water was used to limit aggregating fine particles (<63 µm). Each size 448 fraction was then dried in an oven at ~100°C for 24 hours to remove adsorbed water. Samples 449 were then dry sieved to ensure the accuracy of the wet sieve process. Care was taken to avoid 450 fine particle loss through dust clouds formed during the sieving process. Mass fraction was 451 provided as a function of an equivalent diameter assuming spherical shape, in whole ϕ bins, 452 where $\phi = \log_2(\text{diameter in mm})$, from -2 to >5 (i.e., <0.032 to 4 mm). 453 Representative splits of ~200 particles per size fraction >0.125 mm at each sample site 454 were analyzed for componentry under optical microscope and using a Scanning Electron 455 Microscope (SEM) for smaller particles. Each particle was categorized as one of three 456 components: fresh volcanic glass, older volcanic, and foraminiferans. Lithics and 457 microcrystalline particles were categorized into older volcanics. Micro-textural analysis was 458 continued under SEM. 459 460 461 **Methods References** 462 Hourdez, S. & Jollivet, D. CHUBACARC cruise, RV L'Atalante. (2019). 1. 463 doi:10.17600/18001111 464 465 466 **Data Availability** 467 Videos, photographs, and dive logs from cruise TN401 are available upon request through the 468 National Deep Submergence Facility's archive at Woods Hole Oceanographic Institution. 469 470 471 Acknowledgments 472

- We are very grateful to the Kingdom of Tonga for permission to work in their waters. We thank
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- 479

480 Author contributions

- 481 R.A.B., M.C., S.M.A., D.D., V.J., T.B., J.B., S.B., M.B., L.B., A.C., E.J.C., M.H., M.H., T.M.L.,
- 482 C.Q.P., L.N.R., A.T., and C.M.Y. conducted video and ash thickness surveys via remotely
- 483 operated vehicle. M.C. collected all ash samples, and M.C. and T.G conducted all analyses on
- 484 ash samples. R.A.B., A.C., V.J., and D.D. did post-cruise assessment of video footage and
- 485 comparison to previous cruise footage. R.A.B. wrote the first draft of the manuscript and all486 authors edited the manuscript.
- 487

488 The authors declare no competing interests.

489

490 Additional Information

- Supplementary Information is available for this paper. Correspondence and requests for materials
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- 492 should be addressed to Roxanne Beinart, <u>rbeinart</u>, <u>r</u>
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We would like to thank the reviewers for their helpful suggestions on improving our manuscript. We have now addressed the two major points made by reviewers regarding the addition of quantitative analysis of the animal communities and restructuring some of the text.

We have added an analysis of the community change before and after the eruption using a quantitative, categorical approach. With this data, we were able to show the loss of chemosymbiotic mollusc species from the site with the greatest ash depth, more limited change at a site with less ash, and little change at a site with no ash. We have now provided a figure in the main manuscript for the chemosymbiotic, foundation taxa, and supplementary figures for all other taxa.

We have also addressed all the reviewers' specific points, for which we give detailed responses (blue text) below.

Sincerely, hoxanne Bernout

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REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

This is a fascinating, well-written manuscript, which we enjoyed reading. These sites provide an exciting opportunity to examine how hydrothermal communities respond to substantial ash sedimentation, and highlight the selectivity of this event on species mortality. At present, the manuscript is primarily descriptive; quantitative biological data, such as population counts or areal extent metrics, would help to illustrate the scale of mortality and highlight the fundamental shifts in taxon abundance with statistical significance, but we appreciate that due to the remoteness of the sites, such information may not be available.

The argument for oxygen availability as a primary driver for mortality is intuitive, but other drivers, such as stress-induced chemosymbiont loss, may better explain the patterns of mortality observed. We have included a brief discussion of this alternative hypothesis, which is comparable to coral bleaching, where symbionts are lost due to protracted environmental stress to the host. This appears to be consistent with the preferential loss of chemosymbiotic organisms in the studied sites. We hope that consideration of this alternative hypothesis will be beneficial to the manuscript, at the very least providing a comparative model against which to test the idea that oxygen availability is the primary driver of mortality.

This manuscript will undoubtedly promote future studies into the recovery of communities following major volcanic eruptions, both in modern settings and in the geological record. We hope that the comments below are helpful to the authors going forward, and we would be happy for you to contact us to discuss any of these points further.

1. Data, statistics, and sedimentation

This manuscript documents changes in vent community composition and structure arising from the Hunga Tonga Hunga Ha'apai volcanic eruption. While the authors provide a wealth of supplementary ROV videos and figured comparisons of sites before and after the eruption, it is left to the reader to assess the significance of the changes in faunal distributions. Quantitative data – either rough censuses or areal coverage – would help to illustrate any decreases in specific taxa, and would provide a means to test the statistical significance of the relative changes in faunal makeup whilst minimising subjectivity. The segregation or aggregation of taxa may even provide insight into the spatial patterns of mortality in the communities, relating to the distribution of sediment at the time of deposition. This could in turn permit distinction between smothering by gradual ash accumulation via slow ash fall, versus rapid burial via sedimentary flows, if such spatial data are feasible to derive from the ROV dataset.

As an aside, the presence of large anemones in some of the images (presumably too large to be colonising the area since the eruption?) is of interest. Do you consider them to have survived the event? If so, this could indicate gradual ash fall as a more likely mechanism of sediment delivery. Future searches for sedimentary structures within the deposits would provide a straightforward way to independently distinguish between possible sediment delivery mechanisms.

Based on literature regarding anemone burial, anemones are, perhaps, surprisingly tolerant to sedimentation (e.g., Hendrick et al., 2016, doi.org/10.1371/journal.pone.0149114). This is attributed to their ability to withstand hypoxia and escape from burial. Here, they might have also been helped by the fact that they are commonly found on more vertical surfaces, where sediment accumulation was lower. We have now added text to the manuscript specifically about anemone survival, see lines 189-192.

2. An alternative kill mechanism

The suggestion that the observed patterns of diversity loss can simply be explained by the combined effects of ash burial and taxon-specific oxygen limitations is intuitive, but there is an alternative explanation. The enhanced gills of chemosymbiont-bearing taxa are not a result of oxygen limitation for the host, but rather they are designed to enhance chemosymbiont metabolite uptake (Childress and Girguis, 2011; <u>https://doi.org/10.1242/jeb.049023</u>). During periods of environmental stress, chemosymbionts may be culled by the host to provide autotrophically-derived carbon to supplement the host's diet, or to prevent breakdown of the mutualistic host-chemosymbiont relationship (Reynolds and Rolff, 2008; <u>https://doi.org/10.1186/jbiol88</u>), or they may simply be lost due to decreasing gill size with host starvation (Elisabeth et al., 2014; <u>https://doi.org/10.1186/s12864-019-5456-0</u>), accumulate more toxic chemicals (Bojar et al., 2023; <u>https://doi.org/10.1186/s12864-019-5456-0</u>), accumulate more toxic chemicals (Bojar et al., 2023; <u>https://doi.org/10.1111/1574-6941.12366</u>). Effectively, this could feasibly result in an outcome comparable to coral bleaching, where the stress response of the host (loss of symbionts) invariably results in further stress and enhances mortality.

Of the three dominant taxa in this study, Alviniconcha exhibits the greatest dependency on chemoautotrophy -

with metabolite uptake rates comparable to Riftia, corroborated by nitrogen isotopes (Henry et al., 2008; <u>https://doi.org/10.1016/j.dsr.2008.02.001</u>), and a highly atrophied – though functional – stomach (Laming et al., 2020; <u>https://doi.org/10.1186/s12983-020-00357-x</u>). Bathymodiolus retains a gut and filter-feeding capabilities and supplements its predominantly autotrophic mode of life via heterotrophy (Dubilier et al., 1998; <u>https://doi.org/10.3354/meps165187</u>). Ifremeria exhibits the least dependence on autotrophy, with nitrogen isotopes indicating occupation of a higher trophic niche than Alviniconcha (Henry et al., 2008; <u>https://doi.org/10.1016/j.dsr.2008.02.001</u>). Nonetheless, when unstressed, Alviniconcha, Ifremeria, and Bathymodiolus are all primarily autotrophic. When stressed, Bathymodiolus gradually lose their chemosymbionts during limited sulfide availability (Kádár et al.,

2005; https://doi.org/10.1016/j.jembe.2004.12.025), possibly via lysis (Piquet et al.,

2022; <u>https://doi.org/10.3389/fmars.2022.968331</u>). Such behaviour is also observed in Codakia orbiculata, with rapid lysis of chemosymbionts upon depletion of sulfur granules within the gills (Elisabeth et al., 2014; <u>https://doi.org/10.1111/1574-6941.12366</u>). Chemosymbiont lysis could not sustain C. orbiculata, with significant gill tissue loss (~50%) within 6 months (Elisabeth et al., 2014; <u>https://doi.org/10.1111/1574-6941.12366</u>). Chemosymbiont lysis could not sustain C. orbiculata, with significant gill tissue loss (~50%) within 6 months (Elisabeth et al., 2014; <u>https://doi.org/10.1111/1574-6941.12366</u>). During periods of stress, Alviniconcha also undergoes chemosymbiont loss, though via physical expulsion of mats from the pallial cavity (Sigwart and Chen, 2018; <u>https://doi.org/10.1086/699326</u>). The effect of stress on the chemosymbionts hosted by Ifremeria is not well-known, though evidence of cannibalism – and thus heterotrophy – in captive specimens (Henry et al., 2008; <u>https://doi.org/10.1016/j.dsr.2008.02.001</u>) hints at a similar departure from reliance on autotrophy during periods of stress. This is, however, purely conjecture. It seems from the data presented that Alviniconcha, with the greatest chemosymbiont dependence (Henry et al., 2008; <u>https://doi.org/10.1016/j.dsr.2008.02.001</u>), was amongst those most profoundly affected by the Hunga Tonga Hunga Ha'apai eruption at your studied sites.

The impacts of chemosymbiont loss are significant and wide-ranging; in Bathymodiolus, chemosymbiont loss results in a heightened immune response and enhanced apoptosis within gill cells (Détrée et al., 2019; <u>https://doi.org/10.1186/s12864-019-5456-0</u>), suggesting susceptibility to pathogens. Additionally, chemosymbionts provide an important organic carbon source for the host; though most chemosymbiont-bearing taxa retain a certain degree of heterotrophic capability (Dubilier et al.,

1998; <u>https://doi.org/10.3354/meps165187</u>), they exhibit evidence of starvation without chemosymbionts (Elisabeth et al., 2014; <u>https://doi.org/10.1111/1574-6941.12366</u>). Chemosymbionts in Alviniconcha and Ifremeria (and likely Bathymodiolus) also catalyse the oxidation of reduced arsenic species, decreasing the toxicity of such compounds to the host (Bojar et al.,

2023; <u>https://doi.org/10.1016/j.chemosphere.2023.138258</u>). With chemosymbiont loss, the host organism will be exposed to a greater proportion of reduced arsenic species, dramatically increasing arsenic toxicity.

During periods of enhanced sediment flux, Bathymodiolus would likely exhibit valve closure (Nerlović et al., 2011; <u>https://doi.org/10.2478/s11756-011-0121-3</u>), generating a microenvironment within the valves of the shell. Alviniconcha and Ifremeria both possess opercula (Laming et al., 2020; <u>https://doi.org/10.1186/s12983-020-00357-x</u> and Bouchet and Waren, 1991; <u>http://pascal-</u>

francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=19578750 respectively) capable of minimising sediment influx. Without modulation of chemosymbiont metabolic rates, generating such closed systems would result in rapid depletion of metabolites to the detriment of the host and its chemosymbionts (e.g. Raulfs et al., 2004; https://doi.org/10.1017/S0025315404009087h). During periods of intense nutrient stress, chemosymbionts may transition toward heterotrophy, favouring metabolism of the host's tissues (Piquet et al., 2022; https://doi.org/10.3389/fmars.2022.968331). Such a breakdown of the chemosymbiont-host relationship would necessitate a significant immune response (Reynolds and Rolff, 2008; https://doi.org/10.1186/jbio188) and apoptosis of bacteriocytes (Zheng et al., 2017; https://doi.org/10.1111/mec.14160) to mitigate damage to the host. Here, host-chemosymbiont integration is insufficient to permit host adjustment of chemosymbiont metabolic rates, suggesting that the sole control on chemosymbiont metabolite uptake is via host-mediated chemosymbiont lysis (Zheng et al., 2017; https://doi.org/10.1111/mec.14160). Given that hemipelagic ash settling was recorded for two months post-eruption (Wei-Haas, citation 13), protracted exposure to suspended ash was a near-certainty for all taxa at these sites. Simultaneously, decreased heterotrophic efficiency due to

ash dilution of bioavailable particulate matter would increase the rate of chemosymbiont lysis within the bacteriocytes to mitigate shortfalls in energy availability. These processes would be expected to have a profound impact on chemosymbiont numbers, dramatically reducing the availability of autotrophically-derived carbon and energy to the host. This may be reflected by shifts in carbon isotope fractionation or observed by taking histological sections through gill tissues, and is therefore potentially testable in the future.

We thank the reviewer for this very impressive literature review, but we respectfully disagree that symbiontloss, and, thus, starvation, is an alternative hypothesis for the mass mortality of the chemosymbiotic animals. We did observe some chimneys with chemosymbiotic molluscs at their bases that could have suffered stress associated with symbiont loss before their demise, but we still think that, even if symbiont loss was the proximate cause of death for snails, the initial cause of symbiont loss was likely due to stress induced by the respiratory effects of ash. Though even at sites with heavy ash, the survivors we collected had gills with similar coloration and weight to sites with little to no ash (data not presented here), suggesting a normal symbiont density. Additionally, even if symbiont loss occurred, *Bathymodiolus* mussels that have lost their symbionts can survive for months unfed (Piquet et al., 2022; <u>https://doi.org/10.3389/fmars.2022.968331</u>), which is consistent with molluscs' general ability to withstand months of starvation. We have now added additional text to the manuscript discussing this on lines 175-188.

3. Minor comments

1. 99. Could you provide a little more detail of these ongoing analyses?

We have added "*physical and geochemical*" to the description to clarify what types of analyses are ongoing. Ongoing componentry of <63 um particles and micropaleontological interpretations of biological populations are outside the scope of this paper and will be included in another forthcoming manuscript.

1. 109. Perhaps clarify whether these dilute turbidity currents are being invoked to explain the sedimentation at these sites.

We have clarified submarine density currents as the most likely mechanism to explain sedimentation on lines 109-115. This is the focus of other work so further details have been limited.

1. 128. Perhaps discuss these mats further – do they represent the first stage of succession? Or just a short, opportunistic occupation?

We have amended this sentence and added an additional point that these mats are consistent with the early successional stages observed post-eruption in other vent systems (see Marcus et al., 2009, 10.1016/j.dsr2.2009.05.004). (now lines 138-141)

1. 145-162. See section 2 of this review. Is it possible to test between these hypotheses?

Addressed above in response to Section 2.

1. 155. What is the taphonomy of these un-buried empty shells? Were they always exposed, or could currents have re-exposed them by re-suspending the ash?

In some cases, the shells were found in or near venting fluid, where they are likely exposed due to the actively emitted fluids clearing the deposited ash away. In other cases, the shell hash was surrounding chimney structures, so we interpret this as likely due to dead molluscs falling down onto the sediment surface from vertical surfaces, like hydrothermal chimneys. We have added text to lines 175-180 to clarify this.

1. 170. Could you elaborate on which aspects of vent fluid chemistry have shifted here? (i.e. are there any specific elements that are more or less important?)

The papers cited here describe changes in hydrogen sulfide concentration over time (before and after eruptive activity) at the Eastern Pacific Rise, that is linked to changes in biological communities. We have amended this sentence to now read "to milder perturbations caused by temporal changes in the concentration of the chemosynthetic reductant hydrogen sulfide in venting fluid", see lines 206-207

1. 191-192. Or, reproduction of the few (<20) surviving taxa! If this is the case, early colonisation of Tow Cam by a very genetically-restricted group of descendants would be expected to yield increasing genetic diversity and gene flow through time.

We agree with the reviewers that genetic changes in the population could occur due to this extreme population bottleneck, and an assessment of this is currently underway in the Beinart lab. However, given the survivor population size is extremely small at Tow Cam, we think it is unlikely that they will be a major contributor to recovery, if it occurs, though it cannot be excluded as a possibility. We have modified the sentence slightly to reflect this, now saying "*recolonization via larval supply from distant vents is the MOST LIKELY pathway for recovery for the decimated communities at Tow Cam*"

Figure 1: Your figures and ROV videos are excellent! Just a passing thought – are the Globigerina-like foraminifera likely to have rained into the deposits post-eruption, or are they also victims of the eruption? Could the vertical density currents suggested to have entrained the ash also have entrained foraminifera?

The foraminifera are unlikely to have been deposited after the eruption. An alternative hypothesis is, as you have stated, is entrainment through vertical density currents via ash fall. Identification of the foraminifera and composition throughout the core samples is the focus of alternate work soon to published and likely to shed light on this matter, as, if the foraminifera were entrained, we would expect them to be from pelagic taxonomic groups. However, this analysis is outside the scope of this manuscript.

Figure 2: It would be helpful to state the organisms comprising the microbial mat in b) in the caption. In d), are the anemones survivors of the event, or recolonisers? If the former, it demonstrates remarkable resilience of those specific organisms that might be worthy of note in the paper.

While we cannot determine the exact taxonomic composition of the microbial mat, as identification of prokaryotes cannot be done with visual inspection alone, we have added a statement in the Figure 2 caption that says they are likely sulfur-oxidizing bacteria. As for the anemones, as discussed above, we have now added additional text discussing their survival.

Figure 4: How much sediment do you think has been remobilised and resuspended/removed from the sites in the period between eruption and filming? Is that likely to have impacted the results of this study?

Benthic currents were minimal at most sites. Deposits on seafloor also present limited evidence (ripples) of remobilization following eruption induced deposition. It is unlikely to have any significant effect on the results in the ~3 months following eruption.

1. 440: Perhaps consider including a statement on how much, if any, fine particulate material would have been lost during the sample scooping process.

We believe the volume of fine particulates lost during the scooping process is very limited. Initially a vacuum method was attempted which resulted in a large amount of fines loss, but the bag and coring methods ultimately adopted, resulted in limited fines loss based on visual observation and grain size distribution.

Best wishes,

Reviewer #2 (Remarks to the Author):

This manuscript presents exciting new work on the effects of volcanic ash deposition on marine hydrothermal vent communities. The authors took advantage of an unprecedented opportunity to study the effects of ash deposition on marine communities following a large volcanic eruption, and the work should be of interest to a wide variety of workers in the fields of marine biology, ecology, geology, geochemistry, and paleontology. It is also exceptionally well-written, clearly organized, and sets a clear foundation for future work on the subject. Therefore, it is the opinion of this reviewer that the manuscript is appropriate for publication in Nature Communications Earth & Environmental Science.

The paper presents clear qualitative evidence that benthic marine communities can experience mass mortality as a result of a volcanic eruption on the order of 80-100 km from the provenance, and appears to show variable survivability of such an event. However, it would be greatly strengthened by the addition of a more quantitative analysis of the data. A quantitative analysis would make this study more readily comparable to previous and future studies, and strengthen the core claims of mass mortality and variable survivability of marine taxa following a large-scale volcanic eruption.

I have provided additional comments on a .pdf of the manuscript to be considered at the discretion of the author and editor, and will include some citations below which may be of interest to the authors:

Crawford, R.S., Casadío, S., Feldmann, R.M., Griffin, M., Parras, A., Schweitzer, C.E., 2008. Mass mortality of decapods within the Monte Leon Formation (Early Miocene), Southern Argentina: victims of Andean volcanism. Ann. Carnegie Museum 77, 259–287.

Maguire, E.P., Feldmann, R.M., Casadio, S., Schweitzer, C.E., 2016. Distal Volcanic Ash Deposition As a Cause for Mass Kills of Marine Invertebrates During the Miocene in Northern Patagonia, Argentina. Palaios 31, 577–591.

Maguire, E.P., 2022. The Effect of Volcanic Ash Deposition on Marine Environments, Invertebrate Ecosystems and Fossil Preservation (dissertation, chapters pending publication)

Feldmann, R.M., Franțescu, A., Franțescu, O.D., Adiël, A., Logan, G., Robins, C.M., Schweitzer, C.E., Waugh, D.A., ... , 2012. Formation of lobster-bearing concretions in the Late Cretaceous Bearpaw Shale, Montana, United States, in a complex geochemical environment. Palaios 27, 842–856

Palópolo, E.E., Kroh, A., Harzhauser, M., Griffin, M., Casadio, S., Carmona, N., 2021. An early Miocene spatangoid assemblage on a submarine volcanic ash dune from Patagonia (Argentina). J. South Am. Earth Sci. 108

Wall-Palmer, D., Jones, M.T., Hart, M.B., Fisher, J.K., Smart, C.W., Hembury, D.J., Palmer, M.R., Fones, G.R., 2011. Explosive volcanism as a cause for mass mortality of pteropods. Mar. Geol. 282, 231–239

Orr, P.J., Briggs, D.E.G., Siveter, D.J., Siveter, D.J., 2000. Three-dimensional preservation of a non-

biomineralized arthropod in concretions in Silurian volcaniclastic rocks from Herefordshire, England. J. Geol. Soc. London 157, 173–186.

Hyžný, M., Hudáčková, N., Szalma, Š., 2016. Taphonomy and diversity of Middle Miocene decapod crustaceans from the Novohrad-Nógrad Basin, Slovakia, with remarks on palaeobiography. Acta Geol. Slovaca 7, 139–154.

WIESNER, M.G., WANG, Y., ZHENG, L., 1995, Fallout of volcanic ash to the deep South China Sea induced by the 1991 eruption of Mount Pinatubo: Geology, v. 23, p. 885–888

CAREY, S., 1997, Influence of convective sedimentation on the formation of widespread tephra fall layers in the deep sea: Geology, v. 25, p. 839–842

Please feel free to contact me if there are any questions regarding this review.

Comments from PDF:

While true that there are more examples in the fossil record, it's not particularly common (especially in marine environments) given the large time span of the fossil record.

We thank the reviewer for this observation and have modified this sentence to read "*Mass mortality of marine animals due to volcanic ash deposition is PRESENT in the fossil record…*" now line 49.

There are several other useful examples of this; here are some additional citations: Crawford et al., 2008 (annals of the Carnegie) Maguire et al., 2016 (Palaios) Feldmann et al., 2012 (Palaios) Wall-Palmer et al., 2011 (Marine Geology) Orr et al., 2000 (J. Geol. Soc. London) Palopolo, 2021 (J South Am. Earth Sci.) Hyzny et al., 2016 (Acta Geol. Slovaca)

Thank you to the reviewer for providing these, we have now added them to our references here.

How were juvenile vs mature ash particles differentiated? Even ash that is multiple thousands of years old or more can be pristinely preserved, with delicate bubble shards and smooth glass.

Juvenile (fresh) vs older ash is distinguished primarily based on physical characteristics, texture, luster, color, angularity/rounding. In this unique case where the Hunga deposits represent the entire sediment column, it is a far simpler process to distinguish fresh Hunga sourced ash from older Lau ash particles. Details concerning this process has been added to the Methods section.

Yes! Settling rates in vertical gravity currents can be up to 10 X faster than what Stoke's Law would predict for similar grain sizes. This has been confirmed both in the field and experimentally. Here are a couple additional useful references: Wiesner et al., 1995 (Geology) (observed enhanced sed. rates after the eruption of Mt. Pinatubo) Carey et. al, 1997 (experimental work with ash and vertical gravity currents)

Thanks to the reviewer, we have added these references here now.

Can you clarify here if you are hypothesizing that the ash deposited at the vent communities was the result of the PDCs travelling 80-90 km? Did you also consider air-fall as a potential mechanism, or perhaps a contributing factor?

We have edited this paragraph to clarify the interpretations (lines 99-115). Interpretation of transport mechanism at these sites is the focus on ongoing work yet to be published.

I think that the addition of more quantitative data would be very beneficial to this section. A figure showing a quantitative pre- and post- eruption ecological composition/relative abundence of the studied vent communities would be invaluable. If enough data is available from the photographic and video observations to quantify the post-eruption macrofauna community composition, I think it would be worthwhile to do so. Consider following the more quantitative methodology of Podowski et al., 2009. This would be useful for comparing the present study to previous work, and also to future work documenting the longer-term recovery of these ecosystems.

We have added an exhaustive analysis of the community change here now, using a quantitative approach to compare before and after the eruption. The photogrammetric methods used in the Podowski et al. paper would require very specific and non-trivial surveys at the same heading and altitude with the ROV to capture the same locations and create photomosaic images. This was not part of our original workplan for the research expedition, so, unfortunately, we cannot redo these exactly with the video and photo data we currently have. Our approach that is appropriate for the data we have, shows clear quantitative evidence of change across the system. We assessed 14 taxa in total and have provided a figure in the main manuscript for the main chemosynthetic species, and as supplementary figures for all others. Given the nature of the comparisons, we were not able to undertake a direct comparison of individual areas, instead our categorical abundance estimations capture the severity of the event across the different vent sites.

Are you able to quantify the relative abundance of these remaining mega and macrofauna? How do these motile fauna typically interact with the cessile mollusc community which was more heavily impacted (i.e. are the crabs, lobsters, shrimp, etc. preying or scavenging on the mollusc community that was heavily reduced in population)? How might the dramatic decline of the mollusc community effect these predators and scavengers in the longer term? It would be very interesting to examine the relative abundance of predators and scavengers following the eruption. In the fossil record, we have seen evidence of colonization of ash associated with mass-mortality events by infaunal scavengers, which left behind trace fossils, and even fecal pellets inside the fossil corpses of crabs.

At the request of all three reviewers, we have now added a quantitative analysis of the animal abundances before and after eruption at three sites to this paper. For many of the predators and scavengers, we did see greater abundances more frequently post-eruption at some vent fields, but this appears unrelated to ash depth and so may just reflect normal variation in populations.

Is there information on the flow of these chimneys over time (i.e. are they typically stable and vigorously flowing, or did this change post-eruption)? A change in flow from the chimneys could also have an impact on the ecosystem there.

It is very technically challenging to measure hydrothermal fluid flow rates, so, unfortunately, we cannot address the rate of flow, just confirm that it is present.

Are these white microbial mats typical of these vent communities, or is this something that may have appeared post-eruption? It appears that at least some of it has grown on top of the ash.

Yes, this white microbial mat has grown on top of the ash. White microbial mats (representing sulfuroxidizing bacteria) are typical of hydrothermal vents, and they have also been observed as part of the initial post-eruption successional stage in other regions. We have now added some comments about this on lines 138-141 I think that the addition of more quantitative data would be very beneficial to this section. A figure showing a quantitative pre- and post-eruption ecological composition/relative abundance of the studied vent communities would be invaluable. If enough data is available from the photographic and video observations to quantify the post-eruption macrofauna community composition, I think it would be worthwhile to do so. Consider following the more quantitative methodology of Podowski et al., 2009. This would be useful for comparing the present study to previous work, and also to future work documenting the longer-term recovery of these ecosystems.

As described above, we have now added quantitative data regarding the animal communities to the manuscript by opportunistically using the ROV video available. We agree that future work at these vent fields should aim to continue these video surveys, including direct target revisits, to measure recovery and succession over time.

We have also documented probable respiratory distress in crabs buried in volcanic ash (Crawford et al., 2008; Maguire et al., 2016; Maguire, 2022 (dissertation)). They have been observed preserved with their 3rd maxilliped in a gaping position, which is thought to indicate respiratory distress. Some crabs were also proficient at swimming or burrowing, and were found preserved in life position, indicating they became incapacitated by the ash and were unable to dig themselves out. Because of the very fine grain size reported here, it is likely it invaded the respiratory passages of the crabs and lobsters through the Milne-Edwards openings, and they may have experienced respiratory distress as well. It would be interesting to see how the longer-term interaction with ash may affect their health and lifespan.

We have now added some text, referencing your suggested citations, describing the potential for respiratory distress in the crustaceans, despite their seeming relative survival of the event. See lines 194-198.

What might you expect the faunal succession to look like? Are there any relatively nearby soft-bottom communities which could colonize the volcanic sediment? We have seen a lot of bioturbation and trace fossils in ash beds associated with mortality events in the fossil record. In future studies, it would be very interesting to also examine any newly established infaunal communities which may colonize the new soft bottom environment.

The nearest near-bottom communities in "normal" sediment cover are probably 1-2 km away and have not been previously characterized. We agree that it will be interesting to observe if there is infaunal colonization of this ash and compare it to nearby sedimented communities, but unfortunately, we cannot comment on this here.

It would be useful to have a short explanation of what the colors indicate here, as it is not readily apparent in the text.

These colors match the colors in panel a for particle types, but we have now edited the figure caption to clarify this.

Measuring ash thickness in this way implies that there was no sediment on the sea floor in any part of this system. Has it been documented that this was the case? (I assume it was hard-bottom, basalt-dominated, but wanted to make sure this was documented).

Yes, the lack of previous sedimentation was documented in Ferrini et al., 2008 (10.1029/2008GC002047). We have now referenced this in the manuscript.

Is there a way to preserve the stratigraphy with sampling from an ROV (something like a push-core sample)? If so, it would be useful for analyzing the depositional mechanism to see if grain size changes from the base to the top of the column.

We were not prepared to take sediment cores, as it was not part of the original research plan for this expedition. However, we were able to take a very small number of sediment cores using the limited coring equipment we had on hand at a few of the sites. Analysis of these will be included in another paper focusing on ash transport mechanisms.

Line 456: How was fresh vs older determined?

Juvenile (fresh) vs older ash is distinguished primarily based on physical characteristics, texture, luster, color, angularity/rounding. Juvenile ash contains a massive, smooth texture, shiny, dark brown luster, and a lack of rounding. In this unique case where the Hunga deposits represent the entire sediment column, it is a far simpler process to distinguish fresh Hunga sourced ash from older Lau ash particles. Details concerning the distinction has been added to the Methods on lines 519-527.

Lines 456-457 "Lithics and microcrystalline particles were categorized into older volcanics" What was the reasoning for this?

Free crystals and lithics are suspected to not have originated from the crystal and lithic poor flows observed in Seabrook et al. 2022 (10.1038/s41467-023-43607-2) and Clare et al., 2023 (10.1126/science.adi3038). They represent reworked volcanic particles from far older eruptions in the region. Further detail was added to explain this reasoning on lines 523-527.

Line 457 "Micro-textural analysis was continued under SEM"

Can you be more specific about this method and what it accomplished? Also, what type of SEM imaging was used (i.e. backscatter or secondary?)

These details have now been added to the text in the Methods.

Reviewer #3 (Remarks to the Author):

This work touches on the extremely interesting and little studied topic of the dynamics and recovery of hydrothermal communities after natural disturbance. As rightly noted the existing data on this topic are very scarce and concern only a few areas, while in other areas this issue has not been studied at all. Nevertheless, the issue of recovery of hydrothermal communities is very important, not only from a theoretical point of view, but also from a practical one, given the prospect of extracting mineral resources from such areas. The authors managed to record an unusual moment when the hydrothermal communities were covered with volcanic ash, which had not previously been recorded at all. Having data on the state of hydrothermal communities several years before the volcanic eruption, the authors were able to assess the catastrophic changes in communities that occurred after the eruption. These data can be a good starting point for further observations of the dynamics of hydrothermal communities after natural disturbance. The paper presents the very first data concerning the most general visual observations of megafauna. Despite this, these observations are of great value and I would very much like to wish the authors to continue their work and more detailed studies in the future.

I would like the manuscript to be more structured into sections. The present chapter Results and Discussion contains in part paragraphs that are more related to the Introduction or Methods. In turn, the Introduction chapter contains information that should be included in the Results. It would also be better to separate the results and their discussion into different sections. The results should be described in more detail involving quantitative estimates. The authors have written the Discussion well, summarizing and analyzing most of the

existing work on the dynamics and recovery of hydrothermal biotopes. I recommend major revisions for this manuscript.

Thank you very much to the reviewer for their helpful suggestions regarding organization. We have now reorganized some of the text, formerly in the Introduction, to the Results and Discussion. Respectfully, we feel that a combined Results and Discussion is appropriate for this manuscript. We have also added quantitative estimates of the changes in animal abundances now, which was a request made by all three reviewers.

I'll give some specific comments below.

L. 76. Give a title to all subsection of a section «Results and Discussion».

We have now added the subsection title "Thick ash deposits from the Hunga Volcano observed at nearby hydrothermal vents" to the first section of the Results and Discussion.

L. 68-71. It is the main results and they shouldn't be in the Introduction section.

This has now been moved in the Results.

L. 77-80. This needs to be moved to the Introduction.

This has now been moved to the Introduction.

L. 80-83. It's more about methods.

Respectfully, since the Methods section is at the end in the format for Nature Communications Earth and Environment, we feel this is necessary contextual information to include here for the reader.

L. 86-87. This would be better placed in the Introduction.

We feel that it is appropriate here, in the context of this being a combined Results and Discussion section.

L. 97-111. This is not entirely relevant to the biological results obtained in the work. Some information from this paragraph can be inserted into the Introduction. It is also worth giving a subsection in Materials and methods - «Study area».

The mode of ash deposition is relevant to the biology since it would impact how quickly the ash arrived at the site, which could impact survival. We disagree that this should be moved, as we feel it is appropriate to the fact that this is a Discussion section. Given that the Methods are the end of the manuscript, we also feel that a subsection regarding the study area is redundant, since it will be necessary to include this information in the earlier sections of the manuscript for the reader to have the necessary contextual information.

L.115. What species do you classify as macrofauna in your study? I think you are only describing megafauna.

We have removed the word "macrofauna" here and elsewhere in the manuscript.

L.115. Rather you mean not «density», but «frequency of occurrence».

We do also mean density here, as the categorical abundance (a semi-quantitative estimate of density) has changed between categories.

L.131, L. 136. Could you give more numerical estimates for the frequency of occurrence of different groups of animals before and after the eruption at different vent fields?

We have now added a quantitative assessment of the abundance and densities of animal groups at three vent fields before and after the eruption. Direct numerical estimates were not possible given the opportunistic nature of the ROV video.

L.133. Among the species remained around diffuse venting and on chimneys is of particular interest Vulcanolepas buckeridgeia. Firstly, I'm not sure if it can be attributed to «scavengers, predators, or grazers». They rather are filter-feeders. Moreover, it was noted that they are possibly ectosymbiotic with bacteria, farming the bacteria on the setae for food (Chan and Chang, 2018). What do you think may be the reason for the resistance of this sedentary species to the ash fall? The same question is about zoanthids? In the abstract you also need to insert filter-feeders.

We have now added "filter-feeders" here and in abstract. We have a new paragraph where we discuss the barnacles, see lines 192-194

L. 141: Before you wrote about «five active hydrothermal vent fields and one inactive field along the Eastern Lau Spreading Center-Valu Fa Ridge in the Lau back-arc basin». But now you describe only five fields. Why is there no information on the sixth field? What field is inactive? It is better to describe the fields in the subsection «Study area».

While we quantified ash at the inactive vent field (Kilo Moana) and that is included in our Results regarding ash depth and composition, because it was inactive it didn't have significant chemosynthetic communities even before the ash deposition. Here, our focus is on the active vent fields and our observations regarding the chemosynthetic communities.

L. 145. Please, take a look at the article Nakajima et al., 2019 «Clams after storms: the impact of multiple disturbances on seep vesicomyid clams revealed by long-term monitoring». I think it should be included in the Discussion.

Thank you to the reviewer for pointing out this very interesting paper, but respectfully, since it focuses on a chemosynthetic clam from sedimented systems, it is outside of the scope of our discussion.

L. 145. Can ashes have any other negative effect besides oxygen deficiency and mechanical burying? For example, a toxic effect, especially on suspension feeders and filter-feeders?

Yes, this is mentioned in lines 175-188.

L.164. The title of the subsection should be reformulated.

Without clarification from the reviewer regarding what about this subsection title they think should be changed, we are not able to modify it.

L.167. «chemosynthetic biological communities» throughout the text it is better to replace with «chemosynthesis-based communities».

We have changed this.

Supplementary Video 2. What objects are visible in the upper left corner and in the center?

There is a patch of barnacles in the upper left corner, but without further information, we cannot clarify

what in the center the reviewer is inquiring about.

Dear Dr Beinart,

Your manuscript titled "Deep-sea Pompeii: Hydrothermal vent communities buried by Hunga volcanic ash" 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 under the open access CC BY license (Creative Commons Attribution v4.0 International License).

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. In particular, please explain why you have changed how you refer to the eruption as 'The Hunga eruption'. We note that the commonly used term for this eruption is 'The Hunga Tonga-Hunga Ha'apai eruption' and we strongly recommend you follow existing terminology, unless you have specific and valid reasons for the change.

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Best regards,

Emma Nicholson Editorial Board Member Communications Earth & Environment

Joe Aslin Deputy Editor, Communications Earth & Environment https://www.nature.com/commsenv/ Twitter: @CommsEarth

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

We thank the authors for considering our previous review comments, the revised manuscript addresses the majority of our previous concerns. We appreciate the inclusion of abundance analysis in Figure 3, which really adds to the study. Given the plethora of data available in the supplementary files, there could be more discussion of these ecological results in the manuscript: for example, certain taxa appear to have been able to survive in higher density communities than others (e.g. B. septemdierum vs I. nautilei, Figure 3A), while Alviniconcha is apparently completely wiped out at Tow Cam – this could be mentioned in the section regarding community recovery. Additionally, the population increases in ABE and Tu'i Malila further highlight just how devastating this event was for the taxa at Tow Cam. However, we realise that the authors plan further follow-up publications that may incorporate such discussions.

Line 64 – We recommend inclusion of a citation here to preservation of the Ediacaran biota at various global sites, where fossils are frequently found beneath marine volcanic ash deposits, such as at

Mistaken Point Ecological Reserve UNESCO World Heritage Site (see Conception-type preservation of Narbonne, 2005: www.annualreviews.org/doi/full/10.1146/annurev.earth.33.092203.122519).

Lines 175-180 – This is a good clarification. If you have the data for this, are the empty shells a representative sample of the organisms on the chimney, or is there taxonomic/size fractionation? (n.b. we were unable to access the video file that might provide this information, so apologies if that information is obvious in the videos).

Lines 189-198 – If the anemones also inhabited these vertical surfaces (in addition to the barnacles) it would be worth noting here. Figures S5/S6 could be referenced here to emphasise anemone survival and recolonization. Also, do you have any explanation for why the whelks survived?

Lines 229-232 – As a passing thought, do you record any previously absent taxa occupying this ash (aside from prokaryotes)? Or, do any taxa previously observed appear to prefer this new substrate? Given the limited reworking of this ash after several months, this might suggest that this new niche is here to stay, potentially driving changes to population structure...

Figure 1 The order of the panels doesn't seem to correspond to the caption (and panels require label letters). Mentioning in the caption why the componentry has been done only for a subset of grain sizes may be helpful.

Line S139 – The N value is not provided here, please replace "XXX" with the value.

Line 288 – A couple of these references need some very minor formatting – some text is in block capitals, and Linnaean taxonomy in other references should be italicised. Taxonomic names in the main reference list should also be italicised.

We were unable to access files for videos S2, 3, 4, and 7, getting the error "no video with supported format and MIME type found" on multiple machines, so please check that these videos have uploaded correctly.

All other points we raised in our first review has been effectively addressed, and we look forward to reading future papers resulting from this unique long-term study of community recovery from ashfall disturbance.

Reviewer #3 (Remarks to the Author):

The manuscript has been greatly improved compared with the first version. The authors took into account all my main comments. The manuscript has been significantly restructured in accordance with my suggestions and now it's much easier to read. I would especially like to note that the authors conducted the quantitative analysis of the chemosynthesis-based communities structure before and after the volcanic eruption, as required by all three reviewers, which was completely absent previously. To do this, they chose, in my opinion, a very interesting and suitable method, which made it possible to obtain maps reflecting the quantitative distribution of different taxa that could be used as the basis for further objective assessments of the community succession. The chosen method allowed the semi-quantitative description of the communities and mitigated problems caused by the fact that the submersible dives were not initially planned in such way as to obtain quantitative results. The authors used the available material to the maximum and extracted all possible results from it. The addition of quantitative assessments to the work greatly enhanced the quality and significance of this study. Now I think that this is the very interesting and well-done study that can be recommended for publication in Nature Communications Earth and Environment.

REVIEWERS' COMMENTS:

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Thank you for this comment, we have now clarified the differential impact to *Alviniconcha* snails vs. *Ifremeria nautilei* snails and *Bathymodiolus septemdierum* mussels at the Tow Cam site in the Results (Lines 137-138) and also now added text to the Discussion regarding why this might be the case (Lines 180-182) and how this could impact recovery (Lines 240-243).

Line 64 – We recommend inclusion of a citation here to preservation of the Ediacaran biota at various global sites, where fossils are frequently found beneath marine volcanic ash deposits, such as at Mistaken Point Ecological Reserve UNESCO World Heritage Site (see Conception-type preservation of Narbonne,

2005: www.annualreviews.org/doi/full/10.1146/annurev.earth.33.092203.122519).

We have added this citation here now.

Lines 175-180 – This is a good clarification. If you have the data for this, are the empty shells a representative sample of the organisms on the chimney, or is there taxonomic/size fractionation? (n.b. we were unable to access the video file that might provide this information, so apologies if that information is obvious in the videos).

This is an interesting suggestion but we do not have the data to address this.

Lines 189-198 – If the anemones also inhabited these vertical surfaces (in addition to the barnacles) it would be worth noting here. Figures S5/S6 could be referenced here to emphasise anemone survival and recolonization. Also, do you have any explanation for why the whelks survived?

The anemones do not mostly inhabit vertical surfaces. We have added reference to these Supplementary Figures here as suggested, as well as expanded discussion regarding

survival of the worms. We were unable to find literature regarding whelk survival during burial or respiration of this particular group, and so we are not able to comment on why their survival or escape was possible.

Lines 229-232 – As a passing thought, do you record any previously absent taxa occupying this ash (aside from prokaryotes)? Or, do any taxa previously observed appear to prefer this new substrate? Given the limited reworking of this ash after several months, this might suggest that this new niche is here to stay, potentially driving changes to population structure...

This is an excellent suggestion but we did not sample the ash in a way that would allow assessment of benthic infauna. Anecdotally, we did not notice significant large infaunal populations during the sieving process. Future work in this area should assess infaunal communities in the ash.

Figure 1 The order of the panels doesn't seem to correspond to the caption (and panels require label letters). Mentioning in the caption why the componentry has been done only for a subset of grain sizes may be helpful.

We have modified the caption accordingly.

Line S139 – The N value is not provided here, please replace "XXX" with the value.

Thank you for noticing this, we had meant to delete this, as "i" is not referenced in the Table. We have now adjusted this accordingly.

Line 288 – A couple of these references need some very minor formatting – some text is in block capitals, and Linnaean taxonomy in other references should be italicised. Taxonomic names in the main reference list should also be italicised.

Thank you for noticing these details, we have now fixed these errors.

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