Reviewer #1

The authors offer a glimpse into trace metal and microbial protein distrubution in the Northeast Lau Basin, showing impacts of this basin on transport of important metals into the southern Pacific Ocean basin. Though background microbial populations were similar to the distal plume locations, there were significant increases of differentially expressed proteins belonging to metal transport and chemoautotrophy that were very interesting to detect. The manuscript is incredibly well written and referenced, and I have no comments on what should be corrected except a minor gramatical redundancy (line 27: "comparatively abundant compared"). My expertise is in microbial community/expression analyses, so I do not have the background to review the details of methodology of the trace metal section. I would highly recommend publishing this manuscript as is.

We thank this reviewer for their positive feedback on our manuscript. The typo in the abstract has been corrected.

Reviewer #2

In this manuscript, Cohen et al conduct a systematic sampling of different stations in the pelagic ocean along a transect in the south Pacific Ocean to study trace metal distributions and their potential association with microbial physiology. Trace metal concentrations were measured and observed patterns are associated with both ocean scale and local oceanographic and biogeochemical processes. Additional metaproteomic (and 16S/18S rRNA analyses) were conducted to characterize the microbial (eukaryotic and prokaryotic) communities and the proteins. I found the methods to be solid and the paper to be exceptionally well-written and easy to read. I really commend the authors for a very comprehensive piece of work. Overall, in my opinion, this study represents an important piece of work that expands upon the growing body of trace metal studies across the oceans, and specifically contributes to our understanding of the distribution of dissolved metals across ocean depths and regimes, and their association with microbial metabolism.

I do have some concerns/suggestions for improvement that I outline below.

We are grateful for the detailed comments on the biological portion of our analysis.

I understand that the authors cannot pinpoint exact sources of trace metals since specific hydrothermal vents were not sampled. This is highlighted in the text very briefly – I think it would be useful to have a map showing the 135 known vents in the NE Lau Basin as they can help the user interpret the data and findings better.

Nearby hydrothermal vents are depicted in Fig. 5C (shown with green stars) using the InterRidge Vents Database (Beaulieu et al., 2013), with additional vent coordinates from (Baker et al., 2019). This has been clarified and highlighted for readers in the discussion.

Methods – For the biological analyses are concerned, the only drawback I observe is the Cohen et al 2021 is referred to for most methods. I suggest including some brief additional details here – such as how was the translational metatranscriptome generated? How was the 16S rRNA analyses done?

Details regarding the metatranscriptomic processing and amplicon sequencing analysis have now been included in the methods.

“A translated metatranscriptome was used as the protein database (Cohen et al., 2021). Briefly, the metatranscriptomic data was generated by extracting RNA from 3–51-µm size fraction filters, purifying RNA, removing ribosomal RNA, converting RNA to cDNA and amplifying, and fragmenting to 200 bp. Libraries were sequenced on the Illumina HiSeq platform, and raw data is available through National Center for Biotechnology (NCBI) under Bioproject PRJNA555787. Bioinformatic processing consisted of adaptor trimming, de novo assembly, open reading frame (protein) prediction, and read mapping (Cohen et al. 2021). Taxonomic and functional annotations were performed using the custom-built database PhyloDB, which includes marine prokaryotic and eukaryotic references (https://github.com/allenlab/PhyloDB), and additional iron oxidation, reduction, storage and acquisition annotations were assigned using FeGenie (Garber et al., 2020).”

“Taxonomic composition was further assessed using 18S and 16S ribosomal RNA (rRNA) amplicon sequencing from the 3–51-µm filter size fraction (Cohen et al. 2021). The V3–V5 and V9 regions were targeted of 16S and 18S rRNA fragments, respectively, and sequenced using the Roche 454 platform. The full cDNA prep and bioinformatic processing details are described in Bertrand et al. (2015). The 16S rRNA OTUs were taxonomically annotated using the SILVA rRNA database (release 111) (Quast et al., 2013), and 18S rRNA OTUs using the Protist Ribosomal Reference v.4.11.1 database (Guillou et al., 2013). Principal coordinate analysis (PCoA) of OTU data was performed using Bray-Curtis dissimilarity on center-log-ratio transformed values and implemented with the R package phyloseq (McMurdie and Holmes, 2013).”

Figure 1 – I recommend enlarging the maps as the stations are referred to in the manuscript but finding them currently is difficult.

The maps in Fig. 1 have been modified to more clearly present stations and ocean basin context.

Ln 60 – determined*

Corrected

Line 277 – It is unclear to me as to why the Manganese concentrations are so low. Is this unique to this region or is this also observed in other oceanic regions. I ask because Manganese oxidizing bacteria surely also exist in aphotic water columns elsewhere.

The low dMn concentrations measured in the deep South Pacific are not unique to the region, and dMn similarly follows this distribution in the North Atlantic and North Pacific Oceans. In these regions, surface concentrations are elevated and deep concentrations decrease to a minimum of ~0.1-0.2 nM (Bruland et al., 1994; Van Hulten et al., 2017). High surface concentrations are maintained by the photodegradation of particulate Mn oxides in the presence of organics (Sunda et al., 1983), but decrease deeper in the water column as Mn oxides form, which is partly driven by Mn-oxidizing bacteria. It is likely that the Mn-oxidizing bacteria keep dMn inventories low in the deep ocean, and in regions such as Oxygen Minimum Zones where oxidation is inhibited, both dissolved Mn and cobalt (Co) (which adsorbs onto Mn oxides) accumulate (Johnson et al., 1996; Saito et al., 2017).

Metaproteomics – For the metaproteomics analyses, 20 background samples are used but only a single hydrothermal sample is used. Is there a possibility that more samples could be used? Alternately, could there be some justifications added for this…

We agree that additional samples from the distal hydrothermal plume would strengthen the analysis. However, as described in the beginning of Section 3.3 (see below), real-time tracking of the plume was not performed on the ship, and it was not known that the core of the distal plume had moved since the original observation two decades before. This is therefore a descriptive analysis that can be used to generate future hypotheses, and additional studies are required to confirm metabolic differences between distal plume vs. background waters.

"Since hydrothermal vents were not the focus of this expedition, real-time instrumentation for tracking hydrothermal signatures was not onboard the ship. Instead, seawater samples and biomass was collected at the same location where hydrothermal activity had been observed previously, at St. 12 (Lupton et al. 2004), and analyzed back in the laboratory. We therefore were unaware that the largest metal signatures were at St. 13, or that present-day plume maxima at St. 12 were at 1,200 and 2,200 m, and so biomass was not collected at these depths. However, biomass *was* obtained in the vicinity of hydrothermal influence, at St. 12 (15°S, 173.1°W) at 1,900m, where dissolved and particulate metals were higher than background concentrations. Other deep (≥ 200 m) samples collected across the transect served as background, non-hydrothermally-influenced vent sites (n=20)."

Remnant phototrophic metabolism – This is an interesting finding but I do not think it is important or justified enough to be highlighted as a section title. Are the authors confident that this may not have arisen due to any contamination? Are there any studies looking at degradation of proteins from Prochlorococcus? To be honest, I do not doubt this finding (I have seen this myself in other aquatic settings) but I am always looking for additional lines of evidence to potentially explain this.

The "remnant phototrophic metabolism" paragraph has been moved to its own section entitled "Potential vertical transport of surface phytoplankton". We do not believe that a significant fraction of the protein biomass detected on the 1,900 m filter is derived from contamination. The 1, 900 m ("distal hydrothermal plume") pump collected 0.5 µg/L of protein in 178 L, while surface pumps from <100 m collected on average 7.5 µg/L of protein in 354 L (Table S1). For all of the biomass collected on the 1,900 m filter to be surface contamination, approximately 23 L of surface water would be required to pass through. We believe this is unlikely as the pumps are not programmed to turn on while being deployed or recovered back on the ship's deck, and there was no evidence of a pump misfire. The seawater would furthermore need to passively filter, without pumping, through three membranes (51, 3 and 0.2 µm). Other McLane pumps deployed at this station appeared to collect biomass at the correct depths, with surface and deep samples distinct based on transcriptome content (Cohen et al. 2021). Finally, the metaproteomic analysis carried out in this study shows similarities in the community metabolism recovered from 1,900 m and other hydrothermal vent environments, suggesting that the protein signatures are reflective of deep water. Future analyses will benefit from replication at distal plume depths.

"Contamination cannot be completely ruled out, but is unlikely to be solely responsible for the 1, 900 m protein biomass signal, as approximately 23 L of surface seawater would be required to passively move through the 1,900 m filter during vertical transport to produce the protein levels collected (Table S1).

Future experiments will benefit from replicated biomass collection with large volumes filtered, and examinations into the degradation state of proteins at depth."

We could not find information regarding *Prochlorococcus* degradation rates in the deep ocean. However, there is growing support for fresh dissolved organic material (DOM) exported to the deep ocean that is utilized by heterotrophic bacteria similarly as surface DOM (Bergauer et al., 2018; Kirchman, 2018). We have included statements about these points in the manuscript.

Reviewer #3

The work presented by Cohen et al represents a significant contribution to scientific progress in the field of marine biogeochemistry which is within the scope of this journal. The study combines cutting edge metaproteomic with trace metal measurements representing a rare combination of detailed biological and geochemical data which is needed to further progress our understanding linkages between ocean biogeochemical cycles and microbial ecology. Several new ideas are suggested such as deep-sea organisms exploiting gradients in trace metals via metabolic plasticity, differences in transport proteins between distal hydrothermal plume and background samples reflecting utilisation of or protection from metals by deep-sea micro-organisms and the unexpected presence of proteins associated with photosynthetic processes mainly belonging to Prochlorococcus, either as a result of cell maintenance or present as degraded cells.

The scientific approach is valid, however comment the biological analysis is outside of my area of expertise. I have concerns about the use of the internal standard diluted in the elution acid (Indium) as a method of measuring the recovery of metals extracted from samples as they are loaded onto the chelating column. I would like to see further proof that this is a valid method of assessing and correcting for any losses in sample recovery. The pH at which the samples were loaded onto the column should be stated as this is the main control on recovery of metals from a seawater sample. I would also like to know if there were any checks performed on the recovery of metals from the filters? And further detail on the filter blank value used. The data looks oceanographically consistent and results for reference standards are broadly in agreement with consensus values, but I think addressing these points in the methods text more clearly is important for publication. Please see the specific comments on this section.

The data and manuscript are written in a clear and concise manner with appropriate well-presented figures. I have made minor suggestions for improving several figures/tables as well as adding references. In particular I found the results & discussion of the metaproteomic and microbial community data easy to understand and follow despite not being entirely familiar with this analysis. Well written discussions and presentation of this kind of data are key in papers like this one to bridge the disciplinary gap between ocean geochemistry and biology to bring a combined understanding within the ocean research community.

The careful review by Reviewer #3 is much appreciated, and we are grateful for their effort in strengthening the geochemical aspects of this manuscript. We have specific responses to comments below. Importantly, we clarify that our dissolved metals "partial recovery" method using indium is technically a matrix correction, as indium is not introduced to the column prior to sampling binding, and instead is added into the elution acid. For the particulate metal analysis, indium is used as a proper internal standard, and is added to samples before the digestion. This has been clarified in the text.

Specific comments

Line 20: Should this read “long range transport of trace metals”

Corrected

Line 33: There are other studies that can be referenced that describe an overview of nutrient limitation e.g. J.K. Moore et al 2001 or C.M Moore et al 2013

Corrected

Line 55: also reference Saito nature geoscience paper that looks at this

Corrected

Line 58: While Bennet et al is a corner stone citation for this work there are more recent papers by J. Hawkes that use more robust methods.

Corrected

L206: Supor is the brand name, it should mention that they are made of polyethersulferone (PES)

Corrected

Line125: Were the filters rinsed with DI water to remove salts before freezing?

No, filters were not rinsed with DI to remove salts before freezing since the elements of interest do not require salt correction. It is possible low levels of dissolved metals on dried seawater were also included in the particulate analysis. This has been specified in the methods.

Line 134: If the ammonium actetate buffer had a pH of 6 then this would have resulted in a pH lower than 6 during sample loading when the buffer is mixed with the sample with pH <2. Which would explain the sub-optimal recovery of metals. Unless this is meant to say the samples were passed over the column at a pH of 6? Its always useful to check the pH of the waste from the seaFAST system to measure the pH of sample loading onto the column directly.

Regarding the pH during sample loading, we followed the manufacturer’s (Elemental Scientific) default guidelines in which acidified samples are mixed with concentrated and heavily buffered ammonium acetate (4M) of pH 6 at a 4:1 sample:buffer ratio, enabling sample loading onto the column at pH ~6 (Rapp et al., 2017; Wuttig et al., 2019). This is consistent with reports of complete column recovery from Nobias PA-1 resin for Fe, Mn, Ni, Cu, and Zn with a sample pH between 5.5-7 (Lagerström et al., 2013; Sohrin et al., 2008). Unfortunately, the pH of the waste was not checked, but we will do so going forward.

We tested the pH of a 4:1 acidified seawater sample:buffer mixture to simulate the seaFAST buffering step, which produced a pH of 5.5. Metal binding to WAKO resin at this pH shows similar performance to pH 6 for all metals apart from Mn, where binding (and count rates) increase with higher pH (Rapp et al. 2017).

Rapp et al. (2017) report a recovery of approximately 83.1 ± 2.6% for Fe with Nobias resin at a pH of 6.1, close to our assumed matrix correction of ~83% using In. Other metals with close corrections to ours include Cd (85.5 ± 3.1%) and Zn (95.0 ± 3.4). However, that study shows other metals with recoveries slightly larger (Cu: 102.7 ± 3.4%) or much lower (Ni: 38.5 ± 1.4%; Mn: 24.1 ± 0.2%), and if applied to our analysis, would suggest that our final concentrations are either artificially elevated or severely underestimated, respectively. We do not believe this was the case for Ni or Mn, as the Geotraces GSC sample concentrations match consensus values (Table 1). It is possible our Cu concentrations are elevated compared to reference material and previously published studies partly due to underestimation of column recovery. We have included a note about this in the text (see above).

Line 135: Interesting that you used a 1% nitric acid rinse, was this more effective than DI water or dilute ammonium acetate used in other studies?

We did not test the difference in recovery between DI water and 1% nitric rinse solutions. A 1% nitric acid rinse solution was included in an early seaFAST protocol compiled by Kohler (2017), and was logical as we similarly flush between ICP-MS injections using a weak nitric acid rinse solution.

Line 142: Its worth bearing in mind that the Milli-Q water will also carry a small blank however this is still the best way to estimate the blank contribution.

Line147: What was the make/model of spray chamber/inlet system used?

We used a quartz cyclonic spray chamber (Thermo Scientific). This has been included in the text.

L155: This isn't a great way to estimate recovery as most of the In will pass over the resin column at pH <2 as its diluted in the acid, in comparison to the samples when loaded onto the column at pH 6. It also won't account for any metals lost during the rinsing stage.

Wouldn't a better way to estimate recovery be to compare the values obtained for the GSC reference seawater against the consensus values? I appreciate that this only gives recovery for those standards and that the In calculation maybe necessary to calculate recovery for each individual sample, but perhaps a comparison of values obtained for GSC vs consensus values to show whether or not you get a similar estimate of recovery as using the In calculation? This would help to validate the approach of checking recovery in this manner which I haven't seen before. I can see that most of the numbers in Table 1 are higher than the consensus values so it's possible applying this In correction may have over corrected the values (with the exception of Zn which as you mention is more contaminant prone and trickier to measure).

We tested the reviewer's proposed recovery method using Geotraces reference material by subtracting out blanks from the measured GSC sample concentration, dividing by concentration factor, and dividing by GSC consensus values. We obtain the following averaged estimates (n = 3):

Fe: 89 +/- 6%

Mn: 82 +/- 5%

Ni: 81 +/- 8%

Cu: 108 +/- 11%

Zn: 76 +/- 19%

Cd: 87 +/- 8%

These are close to the average In matrix correction of 83% in our study, for all metals except Cu. It is possible that labile Cu concentrations increase over time in storage, and the consensus values are not reflective of this. We have included a note about this in the text to more clearly present this possibility to readers.

“It is possible that our higher dCu concentrations are a result of long-term acidified storage (8 years), during which time strongly binding refractory organic complexes could degrade and increase labile Cu (Little et al., 2018). Along these lines, Posacka et al. (2017) determined labile Cu concentrations in non-UV-oxidized seawater samples increase with storage time, with long term sample storage at low pH (>4 years) demonstrating similar concentrations to those UV-oxidized and measured within 2 months. Dissolved Cu has previously been reported as 3.1 nM in the deep southwest Pacific using Nobias-chelate PA1 resin (Takano et al., 2017), whereas the maximum raw dCu concentration we obtained in the southwest Pacific was approximately 4.2 nM. It is furthermore possible that the matrix corrections based on In are not reflective of Cu, which has been demonstrated to show both high (103%) and low (~50%) recoveries from Nobias resin at pH of 6.1 (Quéroué et al., 2014; Rapp et al., 2017). In addition, ArNa+ interferences on 63Cu cannot be completely ruled out given the abundance of Na+ in seawater (Diemer et al., 2002), although a cooled spray chamber was used to minimize such polyatomic interferences. The exact mechanism behind these elevated Cu concentrations is unclear at present, and an intercalibration exercise within the trace metal community using long-term stored seawater would be useful to further understand these offsets.”

Another way to add some reassurance on the data quality here is to add additional columns to Table 1 comparing the average and stdev of deep ocean water concentrations measured on this section to the nearby GP13 section which has similar concentrations of macronutrients in deep waters.

We have included a supplemental figure showing our St. 7, 8, and 9 compared to GP16 St. 36, which is the closest station to ours from the East Pacific Zonal Transect (EPZT) expedition (~ 1,000 km to the east). For example, here is how the deep (5 km) concentrations (nmol/kg) compare between GP16 St. 36 (left) and Metzyme St. 8 (right):

Fe: 0.44, 0.65

Mn: 0.06, 0.13

Ni: 7.62, 8.06

Zn: 7.22, 7.72

Cd: 0.79, 0.85

Due to the potential storage-related issues with Cu, we are not directly comparing with GP16 data. The Fe and Cd concentrations are from John et al. (2017) and (2018) (https://www.bco-dmo.org/dataset/643809), and Zn, Ni, and Mn from the Bruland data set (https://www.bco-dmo.org/dataset-deployment/643427).

Loading pH can be checked by adding the buffer and a sample together in the same mixing ratio as the seaFAST to check the pH and compare that pH to the elution profiles in the paper by Rapp and estimate recovery based on the elution profiles in that paper to see if this agrees with the 83 % In estimate.

L175: these measurements were performed on a collision cell iCAPQ and most of the data from the consensus values were likely measured on a sector field ICP-MS, could interference elements also have caused this?

It is possible $ArNa^+$ interfered with ^{63}Cu detection, which is known to occur with a seawater matrix containing high Na^+ (Diemer et al. 2002), although the column chemistry should remove most of the Na. This has been included as an additional mechanism possibly explaining our high Cu concentrations compared to consensus values.

L180: The sharing of consensus values is designed to intercalibrate lab results. The Cu value in table 1 is 1.36 times higher than the consensus value, if I multiply the previously measured dCu by Takano et al 2017 of 3.1 nM I get 4.2 nM which is what is measured here. Is this not evidence of a systematic offset in either this data or that of Takano et al 2017?

It is difficult to say with certainty which factors are responsible for the Cu offset between our data and previously published values. We believe the long storage time of over 8 years could have played a role in liberating labile Cu (Little et al. 2018, Pasacka et al. 2017). We have found the accurate quantification of Cu can be difficult as consensus values may not reflect long storage times, and the community may benefit from additional methodological comparisons. There has been recent discussion within the trace metal community regarding the accuracy of Cu methods applied to long term archives similar to the trends we have observed here.

L200: Is there a reference for this claim that a fraction is lost even after UV exposure? If the Co data has already been published previously you should state this clearly here.

Regarding our claim that a fraction of Co is lost even after UV exposure, this is an unpublished finding that occurred during GEOTRACES intercomparison efforts. We have not published it since it involved other groups' data, but it is worth working with other authors on revisiting this since increasingly groups with ICP-MS datasets are submitting total dissolved and labile methods that are unverified and often not UV-irradiated making their interpretation and intercomparison difficult. Since these matters don't concern this study, we have deleted the sentence.

L210: Can this procedure not be repeated in the lab by cleaning some more filters and rinsing them with seawater? What is the value from the 400 m OMZ sample used as a blank, this should be stated clearly here. Were there any reference materials dissolved in acid to check recovery of the acid digestion procedure. Encase its useful for future reference I have experienced contamination from Milli-Q water systems in the past as most models have a metal knut where the filter screws into the end of the dispenser and this can rust over-time.

We later determined that soaking acid-rinsed filter blanks in oligotrophic seawater for ≥1 week were effective at removing the background contamination, but this was years after the initial digestions were performed and the original batch of filters were not available. It was unlikely to be MQ contamination as this same MQ was used as seaFAST blanks, which were generally low in metal concentration. Indium was used to check recovery and was added with nitric acid prior to the digestion, serving as both an internal standard and matrix correction. Reference material was not used.

Due to high filter blank contamination, samples corresponding to pFe and pMn minimums largely within OMZs were selected from each of the three ICP-MS runs to serve as "blanks", and primarily to compare to the large pFe and Mn signals at St. 12. As a result these blanks are likely overestimated, making the resulting data conservative, but because particulate material in the upper water column is much higher than at depth, this is a relatively small conservative bias. Estimated "blank" concentrations were calculated by converting from cps to pM and scaling up based on In recovery. They are as follows:

St. 1, 3, 5, 6: Fe blank = 130 pM (St. 1 400 m). Mn blank = 5 pM (St. 5 800 m).

St. 10, 11, 12, 2, 7. Fe blank = 117 pM (St. 2. 450m). Mn blank = 3 pM (St. 2 225 m).

St. 4, 8, 9. Fe blank = 240 pM (St. 4 150m). Mn blank = 12 pM (St. 4 400m).

The text has been corrected to indicate the exact depths in which each blank is derived and estimated concentrations.

Our Milli-Q system is an Element system intended for low metal use. It has also been modified to not include the metal nut on the dispenser system, and instead uses a foot operated teflon tube dispenser.

Note: while calculating particulate metal blanks, we realized our dissolved metal blanks were not scaled to take into account the In matrix correction (they assumed no matrix effects). The corrected blank and LOD values are included in Table 2.

	Fe (nM)	**Mn (nM)**	***Zn (nM)**	**Cu (nM)**	**Ni (nM)**	**Cd (nM)**
LOD (n=12)	0.10 ± 0.11	0.006 ± 0.007	0.65 ± 0.39	0.11 ± 0.23	0.04 ± 0.02	0.0008 ± 0.0005
MQ Blank (n=18)	0.14 ± 0.10	0.006 ± 0.005	0.47 ± 0.22	0.06 ± 0.08	0.04 ± 0.03	0.0006 ± 0.0003

L215: I defer to the experience of the other two reviewers on this part of the analysis as this is not my area of expertise.

Ok

L269: suggest upper is changed to Northern

Corrected

L272: scavenging also includes aggregation of colloidal particles

Corrected

L299: Then shouldn't there also be an increase in dMn in the same area?

Line 299: There is a slight enrichment in dMn at St. 2 at approximately 200 m, where dCo reaches the highest concentrations and coincides with an oxygen minimum (Fig. 2&3). Dissolved Co distributions are more driven by biological influences, whereas the dMn distribution is strongly driven by Mn oxide photoreduction in surface waters and Mn oxidation at depth. More intense dMn signals have been observed elsewhere in OMZs due to inhibited Mn oxidation (e.g., Johnson et al., (1996), Lewis and Luther (2000).

Fig 3: Add latitude to Zn* plot, interesting that there doesn't appear to be an increase in Zn* at stn 1 associated with Lohi signal as Roshan et al observe in their paper on the east pacific rise plume but this is observed at station 13. Perhaps a result of the different venting styles.

Corrected

Line 362: provide reference for vent fluid geochemistry please

Removed

Fig 5b: The text would be easier to understand if the predominant direction of water mass movement at 2000 m as an arrow to the map?

The direction of flow at 2 km has been added to the NE Lau Basin map.

L407: because it would require intense upwelling?

L457: Looks like a polynomial regression could also fit to the Fe data and get a similar or improved r2 value. Either show both or give justification statistically for showing the linear trend fits better for Fe.

The exponential fit would be largely driven by one point, and we do not have enough data points to confidently suggest an exponential relationship, however this figure has been included for reference as a supplemental figure (attached).

"Fig. S6B: The relationship between dFe measured at St. 13 and ^{3}He from Lupton et al. (2004) shown in Fig. 5C alongside an exponential curve fit (in purple). Although the exponential fit is strongly supported by a high R2 value, it is largely driven by one data point."

Table 3. When it says the 3He data is extrapolated do this mean the He was matched the sampling depths up with the metzyme sampling depths using the profile shape? That's fine if so but it needs explaining clearer rather than just saying it was extrapolated. Also are the ratios obtained from regression of integrated Fe and He data or regression though individual sample points? Im guessing individual samples but worth stating to be clear as other studies use both or one/other e.g Resing and Fitzsimmons papers in nature/nature geoscience.

Depths that ^{3}He was measured by Lupton et al., (2004) were matched to the depths of dFe values obtained in this study. No upper water column ^{3}He values were available for 400-800 m, and they were therefore linearly extrapolated. In addition, He concentrations were not available in Lupton et al. (2004) and instead were estimated using nearby concentrations, at similar depths, from Jenkins et al., (2019). The ratios were

obtained from the regression of dFe and ^{3}He individual sample points, not integrated. This has been clarified in the Table 3 caption.

L625: Is there an excess of sulphide relative to metals in Lau Basin vent fluids, or are metals present in greater concentrations in which case a lot of free sulphide might be consumed by metal-sulphide mineral precipitation? This data should be available from the InterRidge data set for the vents on your map.

The InterRide Vents database includes polymetallic massive sulfides identified at vent sites in this region. At Eastern Lau Spreading Center vents, high metal concentrations suggest metal-sulfide formation during vent fluid mixing with background seawater, preventing sulfide oxidation by microbes (Hsu-Kim et al., 2008). If this similarly occurs in the NE Lau Basin, it could reduce sulfide availability to microbes. This has been included in the text.

"Metals and sulfide derived from vent fluid likely formed inorganic metal-sulfide clusters, reducing metal toxicity in the microbial community (Edgcomb et al., 2004) and limiting the bioavailability of sulfide to sulfide-oxidizing organisms, as observed in the Eastern Lau Spreading Center (Hsu-Kim et al., 2008; Sheik et al., 2015). Supporting this possibility, polymetallic massive sulfides have been observed in the NE Lau Basin (Beaulieu et al., 2013; Hawkins, 1986)."

L628: This paper by Hang seems relevant here https://www.nature.com/articles/s41586-020-2468-5

This was an important reference and has been included in the bacterial-mediated Mn oxidation discussion section.

"Lastly, heterotrophic Mn-oxidizing bacteria are a major conduit for Mn oxide precipitation in non-buoyant plumes (Cowen et al., 1990). Multicopper oxidase enzymes responsible for heterotrophic Mn oxidation in cultured hydrothermal bacteria (Dick et al., 2006), however, were not detected in the plume-influenced sample. Lithotrophic Mn oxidation is also theorized to occur in Mn(II)-rich vent fluids (Templeton et al., 2005) and has recently been described for the first time in Nitrospirae bacteria of tap water, which show high 16S rRNA sequence similarity to Nitrospirae from the Lo'ihi Seamount seafloor lava (Yu and Leadbetter, 2020). In this analysis, Nitrosopirae proteins were not enriched in distal plume-influenced seawater (>3 μm fraction), although proteins of this phyla were detected elsewhere in the transect. Genes expressed in Nitrospirae and hypothesized to play a role in lithotrophic Mn(II) oxidation, including outer membrane c-type cytochromes and porin-cytochrome c complexes (Yu and Leadbetter, 2020), were similarly not enriched. It is possible that environmental Mn oxidation proteins differ from those characterized in our reference databases, and are therefore missed during bioinformatic annotations."

L677: might be better to say "similar rather than "rivals"?

Corrected

Technical Corrections

Line 43: include not including?

Corrected

Line321: started sentence with lowercase letter dCd

Corrected

Line 329: upregulation of

Corrected

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