

Biogeosciences Discuss., author comment AC2  
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## Reply on RC2

Natalie R. Cohen et al.

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Author comment on "Hydrothermal trace metal release and microbial metabolism in the northeastern Lau Basin of the South Pacific Ocean" by Natalie R. Cohen et al.,  
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We are grateful for the detailed comments on the biological portion of our analysis.

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.

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- $\mu$ m 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- $\mu$ m 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)."

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

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 Hulst 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).

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 ( $\geq 200$  m) samples collected across the transect served as background, non-hydrothermally-influenced vent sites (n=20).”

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  $\mu\text{g/L}$  of protein in 178 L, while surface pumps from <100 m collected on average 7.5  $\mu\text{g/L}$  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  $\mu\text{m}$ ). 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.

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Please also note the supplement to this comment:

<https://bg.copernicus.org/preprints/bg-2021-96/bg-2021-96-AC2-supplement.zip>