Comments reviewer 2 BG-2019-189

We would like to thank the reviewer for the efforts and input provided, which definitely helped to improve the manuscript. We carefully went through all the comments and suggestions and have adjusted the manuscript according to the comments made. Below we provide descriptions of the adjustments we made, addressing the reviewers remarks.

Note) Line numbers: First original manuscript, *second revised manuscript*

__General comments:__

"The link between the different geochemical parameters is not sufficiently detailed. What does the combination of REE and trace metals really bring to the story? Similarly, the link between geochemical parameters and microbial communities is not sufficiently exploited. For example, one of the major results that should have been discussed is Figure S4, which shows the correlations between environmental variables and classes of microorganisms. It is only indicated that there is "a complex array of community drivers within the plume". Moreover, the authors claim that their study represents a T0 before mining activities, but I am not convinced by the analogy between the 2 types of plumes. Indeed, the geochemical characteristics could be similar, the temperature, density, and microbial communities will be totally different.

The aim of this study was to characterize the T0 state of a hydrothermal plume before it is impacted by deep-sea mining to serve as a baseline study which will aid in monitoring of the impacts of plumes created by deep-sea mining, as the situation after mining can then be compared to a state before mining. The plume is characterized in terms of geochemistry and the microbial assemblages as it disperses away from its source. It was not in the scope of this study to exploit the link between the geochemical parameters and microbial communities as we do not have the means to assess all the chemolithoautotrophic and metabolic processes that are going on. The Figure S4 therefore only serves as an initial result and needs to be further studied in future studies. We do agree that our phrasing on an analogue to a mining plume is inappropriate. We have reworded this in the abstract and in the introduction.

__Specific comments:__

1) ***Title, P1, L1: I am not convinced that the results show the successional patterns of trace metals and microorganisms and I would recommend to remove the word "successional".***

   L1 *(L1)*: Removed "Successional"

2) ***Material and methods, sampling, P6: The sampling strategy seems confusing to me. Why several stations were sampled at the same location? What is the difference between these stations? The differences observed for the same parameter among the stations are not discussed. SPM, trace metals, and the microbial community are not systematically sampled at the same location. For example, stations 37, 38 and 39 were only sampled for trace metals. Is there any explanation why the different depths of each station were not systematically sampled for all parameters? It is indicated that intermittent water samples were taken for nutrients, but no information is reported on Table 1. For suspended particulate organic matter, I assume the authors refer to C/N on Table 1. No information is given for the analyses of nutrients and POC/PON. I understand that coring sites were constrained by the coring substrate, by why was not CTD deployed at each coring site?***

Stations were not sampled at the same location, however they were quite close together to study the small scale variability of the hydrothermal plume, which is why they seem to be at the same spot on the map. The latitude and longitude for each station is added in Table 1.

L146 *(L152-155)*: Added information about sampling: "Depths for sampling SPM were chosen to comprise the largest variation in turbidity measured by the WETLabs turbidity sensor in a vertical profile so that the sensor could be reliably calibrated and readings converted to mgL^{-1}. If possible, trace metal and microbial community samples were taken at the same stations and/or same depth."

We have removed the sentence that additional samples have been taken for nutrients and SPOM as we do not use these samples in our study. The C/N column has also been removed from Table 1.

It is a valid point that no CTD's have been taken at the box core locations. However, as the main focus was to follow the plume along its presumed path no CTD's were taken over the Rainbow Ridge following the box core locations due to time constraints.

3) ***Material and methods, SPM analyses, P7: I would have liked to see the values of blank filters and the associated uncertainties as well as the average percentage they represent. Please write down what SEM and EDS mean.***

Information about the values of the blank and the sampled SPM filters are available at the NIOZ data portal (https://dataverse.nioz.nl/dataverse/doi under DOI 10.25850/nioz/7b.b.s).

L159-L161 *(L168-171)*: Added information about the blanks: "To yield SPM concentrations, the net dry weight of the SPM collected on the filters (average of 0.25 mg), corrected by the average weight change of all blank filters (0.04 mg), was divided by the volume of filtered seawater (5 L)"

L162 *(L171-172)*: Changed "SEM" to "scanning electron microscope (SEM)" and "EDS" to "energy-dispersive spectroscopy (EDS)"

4) ***Material and methods, P7: This section is missing some important information and is much less detailed than the following one. Were the filters acid-cleaned before use? What are the values for the blank filters? Were procedural blank performed? Which certified reference material was used to assess the accuracy of the analyses?***

In L167 *(L178)* it was stated that the filters were acid-cleaned: "acid-cleaned 0.45 µm polysulfone filters"

L176 *(L188-191)*: Added information about the procedural blanks: "Furthermore, ten procedural blanks were performed. Half of them were empty acid-cleaned Teflon vials, the other five contained an acid-cleaned blank filter to correct for the dissolved filters. These blanks were subjected to the same total digestion method as described above".

Information about the values for the blank filters will be available at the NIOZ data archive system.

L178 *(L193-195)*: Added information about the calibration: "The concentrations were calculated using external calibration lines made from a multi stock solution, which was prepared by mixing Fluka TraceCert standards for ICP. Rh was used as an internal standard for all elements."

L178 *(L195-196)*: Added information about the drift: "The machine drift was measured before, half-way and after each series of samples and was monitored by using an external drift solution.

L179 *(L196-200)*: Added information about the precision: "Precision (relative standard deviation (RSD)) of these analyses was generally <2 % for major- and trace metals, apart from ^{115}In where the RSD values generally are between 4 % and 8 %, with maximum values going up to 12.48 %. For REE, the RSD values were generally <3 %, apart from a few measurements where RSD values reached maximums up to 12.48 %."

L178 *(L200-201)*: Added information about the accuracy: "The accuracy could not be determined as no certified reference material was analysed."

5) ***Material and methods, P9: For the biodiversity index, the authors should be consistent along the manuscript. With the name of the index (Shannon-Wiener vs. Shannon).***

Changed it to Shannon-Wiener throughout the entire manuscript. (L342 *(L369)*, change made).

6) ***Water column characteristics, P10: Using the T-S diagram, the authors identified 3 water masses. However, the hydrography of the area is certainly more complex than that, as shown in the article by Jenkins et al. (2015), even if this later study was located further south***

   We do agree that the hydrography of the area is more complex, but we wanted to point out the main differences in water masses where we did the sampling.

   L240 *(L265)*: Changed to: "…, whereby three main different water masses could be distinguished."

7) ***Enrichments of trace metals compared to the ambient seawater, P11: In addition to the enrichments factors, I would have liked to see vertical profiles of the absolute values of trace metals and the range of variations. How was the "clear water" defined?***

   Clear water is defined as the water above the plume. Changed made in L288 *(L313)*: "clear water above the plume" to "above plume water".

   A table with the full geochemical dataset (concentrations in pM, with precision in %) will be made public in PANGAEA when the manuscript is published and is also already available in the NIOZ data portal (https://dataverse.nioz.nl/dataverse/doi under DOI 10.25850/nioz/7b.b.s). We have added a table in the supplement (Table S2) showing part of the (trace) metal and REE data as we compare it to other work.

8) ***Geochemical gradients, P12: Fe was found to be linearly correlated to the turbidity with a R2 higher than 93%. What was the p value? In the text, it is written that the chalcophile elements Co, Cu and Zn are shown on Fig. 6A, but only Cu is shown. Same for V and P for Fig 6B and REEs for Fig 6C, where only V and Y are shown. Similarly, in the text, Mn, Al, Ni, In, Pb, Ti and U are referred to Fig. 6D, while Sn is shown on this figure.***

   L297 *(L323)*: "P-value: $2.2*10^{-16}$"

   Clarified that only one element is shown to illustrate the trend they show.

   L299 *(L326)*: "Fig. 6A for Cu"

   L302 *(L329)*: "Fig. 6B for V"

   L304 *(L331)*: "Fig 6C for Y"

   L310 *(L337)*: added "Sn"

   L311 *(L338)*: "Fig. 6D for Sn"

9) ***L301: the authors state that Zn/Fe ratio is elevated at stations 37, 39 and 44. This is also the case at station 40, and is not discussed in the text.***

L301 *(L328)*: Added: "Furthermore, a high Zn/Fe molar ratio is observed at upstream station 40."

10) ***L302: on Fig 6B the relation between V and Fe indeed looks linear, but the axes are drawn with a logarithmic scale, which means that the relations is not linear but polynomial. The V:Fe ratio is not more or less constant and displays values from 0.005 to ~ 0.0012 (please change also on line 462). It is the same for the REEs.***

This is only the case if one of the axes is transformed. If both axes are transformed to a log-scale the same relationships are there as in the case both axes would be on a linear scale. Only if one of the two is on a different axis the relation would be polynomial.

L302 *(L329)*: Changed to: "…and shows varying element/Fe molar ratios without a clear trend of increasing or decreasing ratios".

L305 *(L333)*: Removed "constant"

L462 *(L497)*: Changed to: "slightly varying"

11) ***Microbial assemblages, P13, L316 (L343): Please replace "above plume" by "no plume"***

Accepted.

12) ***L317 (L344): Please replace "which clustered distinctively from each other and from plume and below plume communities" by "which clustered distinctly from each other and from plume, below-plume, and above-plume communities"***

Accepted.

13) ***L318 (L345): Please replace "sediment and near-bottom water samples have communities that are very dissimilar from the overlying water column samples" by "sediment, near-bottom water, and no-plume samples have communities that are very dissimilar from the overlying water column samples"***

Accepted.

14) ***Univariate biodiversity, P13: Data used for Fig. 10 and Fig. 11 is slightly confusing. In Fig. 10, the value for diversity index in the plume is about 3.5 with SE lower than 0.5. In Fig. 11, the values for samples in each plume vary from less than 2.5 to higher than 4.5. So I am wondering if the value in Fig. 10 corresponds to the average value of the data in Fig. 11 or not.***

The values given are the standard error of the mean and are representative of the values used in figure 11. The only difference is the exclusion of station 13 in figure 10 due to it not being considered a legitimate plume data point.

	Mean	Stdev	SE
Above plume	5.046287	0.180401	0.063781
Plume	3.628347	0.804606	0.242598
Below plume	4.701669	0.162479	0.066332
NB water	5.779412	0.227896	0.080573
Sediment	5.958755	0.098144	0.034699
Station 13	4.564791	0.020111	0.01422

15) ***Plume influence on the water column chemical and microbial make-up (P16-17): A table with the range of variation of the literature values would be useful.***

The tables below are added to the supplement (Table S2).

L400-403 *(L432-435)*: "Our chemical results from Rainbow also match with those of Ludford et al. (1996), who have studied vent fluid samples from TAG, Mid-Atlantic Ridge at Kane (MARK), Lucky Strike and Broken Spur vent sites, i.e. element concentrations were found to be in the same order of magnitude (Table S2)."

Location	Sample	Depth	Fe [nM]	Ca [nM]	Al [nM]	Mn [pM]	V [pM]	Cu [pM]	Zn [pM]	Co [pM]	Pb [pM]	Y [pM]	Reference
TAG	14	3477	56	34	1.4	140	260	980		9	15	3.5	German et al. (1991)
TAG	18	3364	87	39	1.2	140	393	620	205	8		6.7	German et al. (1991)
TAG	19	3392	67	35	1.4		323	760	167	6	11	3.7	German et al. (1991)
TAG	22	3337	192	53	1.6	180	888	15440	512	71	21	8.7	German et al. (1991)
TAG	403T	3340	50		0.52	189	239	1405					Edmond et al. (1995)
TAG	403B	3440	38		0.62	193	174	647					Edmond et al. (1995)
TAG	409T	3081	4		1.06	190	32	40					Edmond et al. (1995)
TAG	409B	3231	5		0.3	339	27	20					Edmond et al. (1995)
Rainbow	SAP05_1	2025	278.8	83.6	0.3	184	1389	2386	287	47.2	24.5	13	Edmonds and German (2004)
Rainbow	SAP06_1	1940	26.4	51	1	144	143	134	178	4.1	19.4	2.3	Edmonds and German (2004)
Rainbow	SAP07_1	2150	18	72.2	3.4	216	98	153		5	24.6	2.4	Edmonds and German (2004)
Rainbow	SAP09_1	2100	128.4	38.6	0.9	45	504	1781	751	43.5	7.2	4.1	Edmonds and German (2004)
Rainbow	27	2077	355.43	700.31	2.15	202.78	1910.64	5355.68	2030.09	117.40	32.97	15.61	This study
Rainbow	42	2209	38.42	446.55	0.04	22.47	205.65	396.33	25.47	15.71		0.97	This study
Rainbow	44	2002	132.73	1605.10	2.14	263.64	894.23	1355.13	729.65	77.71	37.23	10.05	This study
Rainbow	45	2166	171.11	1052.82	1.19	116.28	1213.40	1487.52	81.95	44.95	28.69	12.81	This study
Rainbow	46	2280	139.98	455.14	1.67	129.49	917.24	1195.15	353.27	31.14	26.29	9.99	This study

Location	Sample	Depth	La [pM]	Ce [pM]	Pr [pM]	Nd [pM]	Sm [pM]	Eu [pM]	Gd [pM]	Tb [pM]	Dy [pM]	Ho [pM]	Er [pM]	Tm [pM]	Yb [pM]	Lu [pM]	Reference
Rainbow	SAP05_1	2025	6.830	3.630	1.330	5.190	0.951	0.379	0.823	0.150	0.917	0.196	0.543	0.072	0.418	0.061	Edmonds and German (2004)
Rainbow	SAP06_1	1940	1.180	1.290	0.272	1.117	0.217	0.071	0.203	0.035	0.194	0.038	0.108	0.013	0.093	0.013	Edmonds and German (2004)
Rainbow	SAP07_1	2150	1.540	2.380	0.392	1.563	0.293	0.083	0.225	0.041	0.229	0.044	0.121	0.015	0.091	0.012	Edmonds and German (2004)
Rainbow	SAP09_1	2100	2.300	1.380	0.439	1.788	0.330	0.180	0.294	0.050	0.307	0.064	0.174	0.022	0.137	0.018	Edmonds and German (2004)
Rainbow	27	2077	7.179	4.343	1.389	5.250	1.019	0.498	1.149	0.193	1.285	0.274	0.717	0.093	0.521	0.072	This study
Rainbow	42	2209	0.480		0.124		0.090	0.036	0.102	0.010	0.077	0.024	0.054	0.006	0.043	0.003	This study
Rainbow	44	2002	5.562	3.247	1.160	4.037	0.842	0.302	0.984	0.147	0.956	0.228	0.554	0.068	0.410	0.051	This study
Rainbow	45	2166	6.130	3.305	1.308	4.658	0.979	0.375	1.148	0.187	1.252	0.271	0.694	0.089	0.526	0.072	This study
Rainbow	46	2280	4.884	2.972	1.059	3.839	0.803	0.303	0.933	0.155	0.976	0.205	0.537	0.070	0.415	0.059	This study

16) ***Line 408 (L440): Please specify here what you mean with oceanic water masses.***

We meant the water masses mentioned earlier. Removed the term “oceanic” to avoid any confusion

17) ***Line 411: Please specify what you mean with SUP05***

L411 *(L443-444)*: Added a couple of words to explain that SUP05 is a gammaproteobacteria clade; “…such as the Gammaproteobacteria clade SUP05…”.

18) ***Line 442-443 (L475-477): the authors infer the dependence of sediment dwelling Epsilonproteobacteria on nearby plume precipitates, such as Cu, Zn and Cd, but why only these 3 elements. This should be justified.***

Of these elements it is shown that they fall-out of the plume rapidly (both in this study and in others). Added another reference and context to explain this better.

L442-443 *(L475-477)*: “…, thus we infer a relationship between the sediment dwelling Epsilonproteobacteria with nearby plume precipitates, such as Cu and presumed precipitates Zn and Cd (Trocine and Trefry, 1988).”

19) ***Geochemical gradients with the hydrothermal plume, P19: The high Ca:Fe ratio at station 40 is explained by the non-influence of hydrothermal plume. Please add a reference for this statement***

It is shown in this study that the Ca/Fe ratio is high, as the Fe concentrations are much higher within the hydrothermal plume. Because of this we come up with this statement ourselves. To show another study that shows that the abundance of particulate iron is low in water which aren’t influenced by the hydrothermal plume Michard et al. (1984) is added an a reference.

L483-486 *(L519-523)*: “The high molar ratio at staion 40 would then suggest that this station is hardly or not at all influenced by the hydrothermal plume as the natural abundance of particulate iron is low (e.g. Michard et al., 1984 and this study), whereas station 28, 47 and 49 are, as expected, influenced in more moderate degrees compared with the station directly downstream of Rainbow.”

20) ***Microbial gradients within the hydrothermal plume, P20: The authors state that the dominance of Epsilonproteobacteria is likely driven by the strong chemical enrichment of the plume but when looking at Fig. S4, Epsilonproteobacteria is not within the group that is most strongly positively correlated with trace metals. As I wrote above, this point would be very interesting to discuss as well as the other correlations.***

Looking into such patterns required much more rigorous statistical testing, something we cannot do with the number of samples we have. Furthermore, we are reluctant to correlate continuous data with proportional data (microorganisms) with full confidence of inferring relatable patterns.

Added information in the introduction to better emphasise the aim of this study:

L103 *(L105-109)*: "Whilst mechanic understanding of microbial and geochemical interactions in the plume would have required a different experimental setup, which was beyond the scope of the TREASURE project, this paper aims to contribute to knowledge of geochemical and biological heterogeneity in the surrounding of an SMS site, induced by the presence of an active hydrothermal plume, which should be taken into account in environmental impact assessments of SMS mining."

21) L511-513 (L549-551): This statement is too speculative

L511-513 *(L549-550)*: Altered the language, changed to "These patterns may relate to ecological succession (Connell and Slaytor, 1977) within the plume…"

L513-515 *(L551-553)*: The use of likely probably created a too speculative tone, therefore we changed from "likely" to "possibly". No other hypotheses are put forward.

Figures and tables:

22) Fig. 1: Station 30 is indicated twice.

Changed one 30 to 33.

23) Fig. 2: The x axis represents the distance from Rainbow. On Fig. 1 it looks like station 44 is located closer to Rainbow than station 26

That's because we measured the distances to Rainbow along the transect of the plume instead of its direct distances. Changed the description of Fig. 2 to include that it follows the plume transect as found in Fig. 1 "Transect along main plume path (indicated in Fig. 1 as plume transect), showing turbidity in the water column. The plume is indicated by highest turbidity values and disperses away from the Rainbow vent field."

24) Table 1: Could you indicate long-lat for each station?

Added latitude and longitude for the stations.