

## Comments reviewer 1 (Valérie Chavagnac) BG-2019-189

We would like to thank Valérie Chavagnac for her efforts and input provided. 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 made, addressing the reviewer's remarks.

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

### General comments:

**1) “I thought that the submitted paper will provide key information on the close interaction between microbial diversity and the environmental conditions”**

The aim of this study was to characterise the 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 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 is not in the scope of this study to exploit the close interaction between the microbial diversity and the environmental conditions. We do agree we should have made this clearer at the start of the manuscript and have made adjustments in both the abstract and the introduction.

L21-24 (L21-L24): “Understanding how hydrothermal plumes can be characterised by means of geochemistry and microbiology as they spread away from their source and how they affect their surrounding environment may help in characterising the behaviour of the dilute distal part of chemically enriched mining plumes.”

L36 (L41-43): Added: “This study of a hydrothermal plume serves as a baseline study to characterize the natural plume before the interference of deep-sea mining”.

L103 (L105-109): “Whilst mechanistic 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 surroundings 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.”

**2) *“It lacks general information, some references are missing and the geochemical data are missing”***

Based on the comments given in the rest of the manuscript general information and missing references are added. Please see the comments below for more details.

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.

**3) *“I cannot see their data and how they have been acquired (the methodology is poorly described)”***

We have extended the methodology to better describe how the data have been acquired. The changes are shown at general comments 7 and 8 in more detail.

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.

**4) *Abstract: I find the abstract too vague and not enough information on what the authors have done during the course of their study. I suggest to reduce the first paragraph and to concentrate the text on the results and conclusions.***

We did not reduce the first paragraph as we think it is important information as this study was done within the TREASURE project, which is related to deep-sea mining. However, we have made changes, focusing more on the results and conclusions.

L21-24 (L21-24): Changed “Understanding how natural hydrothermal plumes evolve as they spread away from their source and how they affect their surrounding environment may provide some analogies for the behaviour of the dilute distal part of chemically enriched mining plumes.” to “Understanding how hydrothermal plumes can be characterised by means of geochemistry and microbiology as they spread away from their source and how they affect their surrounding environment may help in characterising the behaviour of the dilute distal part of chemically enriched mining plumes.”

L31-32 (L31-37): Expanded "...the neutrally buoyant plume stood out by its enrichments in (trace) metals and REEs, of which the concentrations changed as the plume aged", to "...the neutrally buoyant plume stood out by its enrichments in (trace) metals and REEs as e.g. Fe, Cu, V, Mn and REE were enriched by factors of up to ~80, ~90, ~52, ~2.5 and ~40 respectively, compared to clear water samples taken at 1000 m water depth. The concentrations of these elements changed as the plume dispersed shown by the decrease of element/Fe molar ratios of chalcophile elements (Cu, Co, Zn), indicative of rapid removal from the hydrothermal plume or removal from the solid phase. Conversely, increasing REE/Fe molar ratios imply uptake of these elements from the ambient seawater onto Fe-oxyhydroxides."

**5) Introduction: As it stands, by the end of the introduction, I don't have any clues on the methods that you will be using and for what. Please provide some additional information.**

We have provided additional information on the methods used.

L97-100 (L101-105): Changed "Geochemical and biological changes were tracked vertically in the water column and horizontally along the neutrally buoyant plume to study the heterogeneity in the background pelagic system that was influenced by the hydrothermal plume." to "Geochemical and biological changes were explored vertically in the water column and horizontally along the neutrally buoyant plume using HR-ICP mass spectrometry to determine the (trace) metal and REE content of the SPM and next generation sequencing methods were used to quantify the heterogeneity in the background pelagic system that was influenced by the hydrothermal plume."

**6) Material and methods, study site: Some information are missing and are provided in German et al., 1996; Marques et al., 2006**

In our opinion not much was mentioned in these papers what we did not mention yet in our setting description. We have added German et al. (1996) and Marques et al. (2006) as additional references (L111 (L116); L114 (L120)).

**7) Material and methods, suspended particulate matter analysis: Unclear on the procedure you applied. What has been done onboard and onshore. Please clarify.**

It was mentioned what was done onboard (L156 (L165)) "The subsamples were filtered on board over pre-weighed 0.4 µm polycarbonate filters." To better emphasize what we did on shore we changed L158 (L167) to "In the laboratory, the filters were freeze dried..."

L163-164 (L173-174): Added under which conditions the SEM was operated: "The SEM was operated under an acceleration voltage of 15 kV and a filament current of 1850 mA."

**8) Material and methods, chemical analysis: Unclear what has been done onboard and on shore. Please provide additional information about the calibration of the instrument, the blank, the drift correction etc. Where is the table of results?**

In order to make it more clear what was done onboard and onshore the following changes have been made:

L166-167 (L178): "...water samples were filtered on board..."

L170 (L181): "Filters were dried in the laboratory..."

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

L178 (L193-195): Added information about the calibration of the instrument: "The concentrations were calculated using external calibration lines made from a multi stick 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 measurements: "The machine drift was measured before, half-way and after each series of samples and was monitored by using an external drift solution."

L178 (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 <sup>115</sup>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 with 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."

L178 (L201-204): Added information on what the blanks were used for and how the true concentration was calculated: "The data of the samples was corrected for the dissolved filters by subtracting the average result of the five blank filters. Subsequently the data was recalculated to account for the dilution of the samples during the total digestion and the amount of seawater that was filtered to yield the true concentration of each element."

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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.

**Specific comments:**

- 1) ***Abstract, P2, L30: "Both vertically in the water column and horizontally along the neutrally buoyant plume, geochemical and biological changes were evident as the neutrally buoyant plume stood out by its enrichments in (trace) metals and REEs, of which the concentrations changed as the plume aged."***

***I find this sentence too vague to provide additional information compared to the literature. It would be much appreciated to add some quantification on trace element concentration for example.***

L31-32 (L31-37): Expanded "...the neutrally buoyant plume stood out by its enrichments in (trace) metals and REEs, of which the concentrations changed as the plume aged", to "...the neutrally buoyant plume stood out by its enrichments in (trace) metals and REEs as e.g. Fe, Cu, V, Mn and REE were enriched by factors of up to ~80, ~90, ~52, ~2.5 and ~40 respectively, compared to clear water samples taken at 1000 m water depth. The concentrations of these elements changed as the plume dispersed shown by the decrease of element/Fe molar ratios of chalcophile elements (Cu, Co, Zn), indicative of rapid removal from the hydrothermal plume or removal from the solid phase. Conversely, increasing REE/Fe molar ratios imply uptake of these elements from the ambient seawater onto Fe-oxyhydroxides."

- 2) ***Abstract, P2, L34: "...the biodiversity appeared to reduce with distance away from the Rainbow hydrothermal vent field"***

***What is this biodiversity change?***

The change in biodiversity of the microbial background pelagic system was that it reduced with distance from the Rainbow hydrothermal vent field. Biodiversity was quantified into a univariate indice to quantify this reduction in diversity.

L34 (L39): changed to "...univariate microbial biodiversity declined with distance away from the Rainbow hydrothermal vent field."

- 3) ***Abstract, P2, L36: What would be the connection with the impact of deep-sea mining?***

L36 (L41-L43): Added: "This study of a hydrothermal plume provides a baseline study to characterize the natural plume before the interference of deep-sea mining".

**4) Introduction, P2, L42: Add reference**

L42 (L49-50): Added Cave et al. (2002) and Chavagnac et al. (2005) as references.

**5) Introduction, P2, L44: Remove possible**

L44 (L51): Removed possible.

**6) Introduction, P3, L58: "Remove south of the Azores", change to "36°14" N on the MAR"**

L58 (L65): Changed "south of the Azores" to "36°14" N on the MAR"

**7) Introduction, P3, L59: "...it ejects one of the most prominent and persistent natural plumes on the MAR"**

***Hydrothermal fluids at Rainbow are extremely enriched in Fe compared to other vent fields along the MAR. However, the substratum is not solely composed of basalt as it is elsewhere such as Menez Gwen, Lucky Strike etc. It would be valuable to provide additional information with some references.***

In the following paragraph of the introduction we mention that it is shown that the host rock influences the hydrothermal fluid composition (see L69-70 (L78-79): "..., that the underlying host rock influences the hydrothermal fluid composition...".) Furthermore, it is mentioned in the setting description that the basement rocks are different compared to most other sites, L113-122 (L118-125): "The vent field, which is approximately 100 by 250 m in size, is underlain by a basement composed of ultramafic rocks (Edmonds and German, 2004). The ultramafic setting of Rainbow is atypical for the region, which is dominated by basalt hosted vent systems (Douville et al., 2002). Due to serpentinization reactions during the circulation of the hydrothermal fluid in the peridotite basement rocks, the Rainbow vent field produced plumes particularly enriched in transition metals (notably Fe, Mn and Cu) and REE (Douville et al., 2002; Findlay et al., 2015). On the contrary the plumes are depleted in hydrogen sulfides (Charlou et al., 1997; Douville et al., 2002), resulting in relatively high metal/sulfide ratios."

**8) Introduction, P3, L62: "The same currents will also disperse mining plumes, created in the vicinity of the hydrothermal vent. These mining plumes are therefore likely to interfere with the hydrothermal plume and thus potentially alter baseline (T0) conditions."**

***I don't really understand what you want to say here.***

L62 (L68-70) Changed to: “Basic knowledge of natural plumes is essential to be able to discern mining impacts consisting of plumes created in the vicinity of the vent during excavation and by discharge of the return flow which may interfere with the natural hydrothermal plume.”

**9) Introduction, P3, L64: “...understanding natural plume processes may reveal how ecosystems adapt to elevated turbidity and co-occurring changes in the chemical environment.”**

***If you look at the hydrothermal plume as it is at the Rainbow vent, then you will define the close link between the biodiversity and the environmental changes. I don't see how you can address the resilience of plume ecosystem to turbidity changes. I don't get the point. Please clarify.***

L64 (L74): Removed the sentence.

We don't want to address the resilience of plume ecosystem to turbidity changes. We want to provide knowledge of hydrothermal plumes in terms of geochemical and microbial community composition.

**10) Introduction, P3, L68: “...the composition of the hydrothermal fluid and the associated sediment formed by precipitation from the hydrothermal plume have been established.”**

***The sediments are not precipitated from the plume but parts of the polymetallic particles formed within the plume are preserved within the sediment. I don't understand your sentence.***

L68 (L76-77): Changed to: “...the composition of the hydrothermal fluid and sediment influenced by fall-out of particulates from the Rainbow and other hydrothermal plumes have been published.”

**11) Introduction, P3, L70: See the work from Marques et al., 2006**

L70 (L79): Added reference to Marques et al. (2006)

**12) Introduction, P3, L72: I have done some work on these sediments, especially on REEs. See Chavagnac et al., 2005**

L72 (L81): Added Chavagnac et al. (2005) as reference. Changed to “...showed enrichments of Fe, Cu, Mn, V, As and P, as well as REE (Chavagnac et al. (2005)), as a result of fallout from the hydrothermal plume.”

**13) Introduction, P3, L73: “...deposition from the plume is partially being influenced by microbial activity which enhances scavenging and oxidation rates...”**

***I don't understand the link between deposition and enhanced element scavenging by microbial activity. Please rephrase***

L72-76 (L81-84): Rephrased to: "It has further been shown that microbial activity influences plume processes (Breier et al., 2012; Dick et al., 2013), such as scavenging and oxidation rates of metals (Cowen and Bruland, 1985; Cowen et al., 1990; Mandernack and Tebo, 1993; Dick et al., 2009),..."

**14) Introduction, P3, L76: What are the implications?**

L76 (L84): Changed to: "...influencing the local ocean geochemistry."

**15) Introduction, P3, L77: Chemolithoautotrophic?**

Yes, changed throughout the manuscript.

L77, 78, 405 (L86, L87, 437): "chemolithoautotrophic"

L565 (L603): "chemolithoautotrophs"

**16) Introduction, P4, L82: See also Borja et al., 2014; Borja et al., 2016; Reed et al., 2015; Orcutt et al., 2011**

We have added citations

L79 (L87): Orcutt et al., 2011

L87-88 (L97-99): "Considering the majority of microbial growth is predicted to occur in the neutrally buoyant portion of the plume (Reed et al., 2015), further efforts should be concentrated on sampling this portion of the plume."

**17) Introduction, P4, L83: "...dilution of vent associated microorganisms..."**

***I don't understand this part of the sentence. Please clarify.***

L83 (L91-92): Changed to "...reduction in dominance of vent associated microorganisms..."

**18) Introduction, P4, L84: "...communities associated with the rising plume would disperse with distance from the vent on a scale of metres, showcasing a variable community within the plume."**

***Unclear, please rephrase***

L84 (L92-93): Changed to "...suggesting that communities associated with the initial rising plume become diluted on a scale of metres."

**19) Introduction, P4, L86: "...dispersed over potentially hundreds of kilometres..."**



***Hydrothermal dissolved iron can be tracked up to 4000 km. See the paper of Resing et al., 2015***

The dissolved part can be traced up to 4000 km, however, this is not the case for the particulate part. Made a change to address this.

L86-88 (L95-97): Changed to: "..., remaining traceable in particulate form to at least 50 km away from its source (Severmann et al., 2004), and even up to 4000 km in dissolved form (Resing et al., 2015).

**20) Introduction, P4, L90: What do you mean by 'chemical fractionation'?**

L90 (L101): Changed "chemical fractionation" to "Geochemical and biological changes".

**21) Introduction, P4, P90: "Notably, due to the lack of quantified characteristics of SMS mining plumes (especially the discharge plume), the T0 influence of this hydrothermal plume may act as an analogue for future mining plume impacts."**

***To date, there are no exploitation deep-sea mining sites (soon in the Pacmanus basin). So I don't understand what you want to say by SMS mining plume, and T0 influence. Please rephrase.***

L90 (L68-70): Rephrased to: "Basic knowledge of natural plumes is essential to be able to discern mining impacts consisting of plumes created in the vicinity of the vent during excavation and by discharge of the return flow which may interfere with the natural hydrothermal plume."

**22) Introduction, P4, L94: "Although it should be kept in mind that discharge plumes will have different physical characteristics as these plumes will have a higher initial density and therefore would tend to sink rather than maintain buoyancy and may have a different release depth."**

***Please provide some references to sustain your text. It is unclear when you refer to natural plume compared to the one generated by deep-sea mining exploitation.***

L93 (L70-71): Changed "discharge plumes" to "mining plumes".

L94 (L72): Added Gwyther et al., 2008 and Boschen et al., 2013 as references

**23) Introduction, P4, L96: Please start with a new paragraph here**

L96 (L100): Started new paragraph.

**24) Introduction, P4, L97: If you track changes then, you know what are the environmental conditions outside the immediate impact of hydrothermal plume? Is it right?**

Yes, in the manuscript we provide comparisons between plume and non-plume influenced waters (i.e. above-plume).

**25) Introduction, P4, L100: "By utilising a range of methods that could be useful as monitoring techniques and describing background environments that may be influenced by SMS mining, we contribute to site specific knowledge of the Rainbow hydrothermal vent plume behaviour, associated (trace) metal enrichments and microbial community composition." Too long. Please rephrase. I suspect that you have specific tools for microbial diversity associated to others more specific to chemical monitoring. Is it right?**

L97-100 (L101-105): Changed to name the specific tools used for the analyses: "Geochemical and biological changes were studied vertically in the water column and horizontally along the neutrally buoyant plume using HR-ICP mass spectrometry to determine the (trace) metal and REE content of the SPM. Next generation sequencing methods were used to quantify the heterogeneity in the background pelagic system that was influenced by the hydrothermal plume."

**26) Material and methods, P6, L135: I don't understand the term gradient. What do you mean? Please clarify.**

L135 (L140): Changed "gradient" to "path".

**27) Material and methods, P6, L138: Which type of CTD rosette? Do you follow the GEOTRACES recommendations? Please explain.**

Although the method applied by us was similar to the GEOTRACES recommendations, it was not completely similar. Concerning the sampling in general, nutrient samples were taken along with all trace element samples to verify the proper bottle and rosette operation and sampling depth (i.e. to compare the hydrography established with the conventional CTD/Rosette). As recommended by GEOTRACES the filtration was done directly from pressurized bottles and the recommended filters and filter holders were used (Pall Gelman Supur 0.45 µm polyethersulfone filters and Advantec-MFS 47 mm polypropylene inline filter holders). The filters were acid-cleaned before used. However, our blanks were acid-cleaned unused filters whereas GEOTRACES recommend otherwise to correct for the absorption by the filter.

L138 (L143): CTD was a Seabird 911 system. Changed in text to “Seabird 911 CTD-Rosette system”.

**28) Material and methods, P6, L140: What do you mean by temporal? This is unclear.**

We don't agree as it is mentioned that CTD casts have been taken continuously over 12 hours, to study the temporal changes (i.e. the changes over time).

**29) Material and methods, P7, L160: “...or once again if the difference between the two measurements was 0.03 mg or more.”**

**Unclear**

L160 (L167-168): Changed to: “...or in triplo if the difference between the first two measurements was more than 0.03 mg.”

**30) Material and methods, P161: Please provide some additional information about the instrumental procedure you used. Standards?**

L161 (L173-174): Added: “The SEM was operated under an acceleration voltage of 15 kV and a filament current of 1850 mA”.

**31) Results, P10, L250: “Against a background of non-plume influenced waters with typical concentrations of SPM of 0.04 mgL<sup>-1</sup> (0.015 NTU)...”**

**Where did you get this information? Is it your data? Or from literature? Please clarify.**

L250 (L275): Added: “..., as found in the CTD casts,..” to clarify how these data were obtained.

**32) Results, P10, L252: “The apparent continuity of this turbid water layer, especially to the NE of the Rainbow field, and lack of similarly turbid waters in the bottom waters below the plume, link the plume to Rainbow and preclude an origin in local sediment resuspension.”**

**This is already the discussion**

L379 (L408-410): Moved text above to discussion paragraph 4.1 “The apparent continuity of this turbid water layer, especially to the NE of the Rainbow field, and lack of similarly turbid waters in the bottom waters in the bottoms below the plume, link the plume to Rainbow and preclude local sediment resuspension as origin.”

**33) Results, P11, L276: The database of geochemical composition is not huge. I wonder whether the statistic treatment is appropriate? Where is the data table?**

It is a non-constrained ordination and not a statistical test per se, there are no p-values. It is a visualisation of the similarity between the samples.

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.

**34) Results, P12, L291: Where is the data?**

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.

**35) Figure 2, P32: How does it compare to the work of German et al., 1996 in this area? If you want to address the temporal change of hydrothermal plume environment, this is one way to compare the neutrally buoyant plume features 20 years apart. That would be great.**

In the discussion we mention the comparison of our results to those of German et al. (1998). L379-381 (L410-413): “Using turbidity measurements and presumed plume path, we traced the plume up to 25 km away from the vent source. This is within the range mentioned by German et al. (1998) who found that the Rainbow plume extends over 50 km, being controlled by local hydrodynamics and topography.”

Furthermore, we have added a table in the supplement (Table S2), comparing part of our data with o.a. German et al. (1991).

**36) Figure 5, P34: It will be interesting to indicate the station? A color coding as in Fig. 6. Did you use the NTU measured at the depth of water collection?**

Changed Figure 5 to include the colour coding

Changed description of Figure 5 to: “Relationship between in-situ measured turbidity and molar concentration of particulate iron.”

**37) Figure 6, P35: “Relationship between copper (a), vanadium (b), yttrium (c) and tin (d) to iron” Geochemical analyses of the waters? Or is it the SPM? Not clear. Data?**

Changed to “Relationships between molar concentrations of particulate copper (a), vanadium (b), yttrium (c) and iron (d) to iron collected from the filtered water samples.

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.

**38) Figure 7, P36: Comparison with the work of Cave et al., 2002 and Chavagnac et al., 2005, Edmonds and German, 2004**

Comparison with work of Cave et al., 2002 and Edmonds and German, 2004 is described in the discussion section 4.3.

L473 (L508): Added Chavagnac et al. 2005 as a reference