~~Successional p~~Patterns of (trace) metals and microorganisms in the Rainbow hydrothermal vent plume at the Mid-Atlantic Ridge

Sabine Haalboom[1,*], David M. Price[1,*,#], Furu Mienis[1], Judith D.L van Bleijswijk[1], Henko C. de Stigter[1], Harry J. Witte[1], Gert-Jan Reichart[1,2], Gerard C.A. Duineveld[1]

[1] NIOZ Royal Netherlands Institute for Sea Research, department of Ocean Systems, and Utrecht University, PO Box 59, 1790 AB Den Burg, Texel, The Netherlands

[2] Utrecht University, Faculty of Geosciences, 3584 CD Utrecht, The Netherlands

[*] These authors contributed equally to this work

[#] Current address: University of Southampton, Waterfront Campus, European Way, Southampton, UK, SO14 3ZH.

sabine.haalboom@nioz.nl; D.M.Price@soton.ac.uk

Keywords: Rainbow vent; Epsilonproteobacteria; Hydrothermal vent plume; Deep-sea mining; Rare earth elements; Seafloor massive sulfides

Abstract

Hydrothermal vent fields found at mid-ocean ridges emit hydrothermal fluids which disperse as neutrally buoyant plumes. From these fluids seafloor massive sulfides (SMS) deposits are formed which are being explored as possible new mining sites for (trace) metals and rare earth elements (REE). It has been suggested that during mining activities large amounts of suspended matter will appear in the water column due to excavation processes, and due to discharge of mining waste from the surface vessel. Understanding how ~~natural~~ hydrothermal plumes can be characterised by means of geochemistry and microbiology ~~evolve~~ as they spread away from their source and how they affect their surrounding environment may

help in characterising ~~provide some analogies for~~ the behaviour of the dilute distal part of chemically enriched mining plumes.

This study on the extensive Rainbow hydrothermal plume, observed up to 25 km downstream from the vent site, enabled us to investigate how microbial communities and (trace) metal composition change in ~~the presence of~~ a natural plume with distance. The (trace) metal and REE content of suspended particulate matter (SPM) was determined using HR-ICP mass spectrometry and the microbial communities of the neutrally buoyant plume, above plume-, below plume-, and near-bottom water and sediment were characterised by using 16S rRNA amplicon sequencing methods. 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 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 above plume water samples taken at 1000 m water depth.~~,~~ ~~of which t~~The concentrations of these elements changed as the plume aged 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 REE from the ambient seawater onto Fe-oxyhydroxides. This was also reflected in the background pelagic system as Epsilonproteobacteria started to dominate and ~~the~~ univariate microbial biodiversity ~~appeared to reduce~~declined with distance away from the Rainbow hydrothermal vent field. The Rainbow hydrothermal plume provides a geochemically enriched natural environment, which is a heterogeneous, dynamic habitat that is conducive to ecological changes in a short time span. This study of a hydrothermal plume provides ~~as~~ a baseline study to characterize the natural plume before the interference of deep-sea mining.

1 Introduction

Hydrothermal vent fields found at mid-ocean ridges and back-arc basins are known for discharging fluids rich in potential microbial energy sources such as H_2, H_2S, CH_4, NH_4 and Fe (Jannasch and Mottl, 1985; McCollom, 2000). In addition, they are characterised by the presence of polymetallic sulfide deposits containing high grades of metals like Cu, Co, Zn and rare earth elements (REE) (Cave et al., 2002;

Chavagnac et al., 2005). Because of the steadily increasing demand for these metals, and their geo-political distribution on land, hydrothermal vent deposits are explored as new ~~possible~~ mining sites (Hoagland, 2010). Since such areas accommodate unique and vulnerable marine life, serious concerns exist about the environmental sustainability of seafloor massive sulfide (SMS) deposit mining (Boschen et al., 2013; Collins et al., 2013), especially with regards to the effects of the different plumes, which are generated during the excavation of ores and by the return flow of wastes in the vicinity of hydrothermal vents (Ramirez-Llodra et al., 2011; Vare et al., 2018). As SMS mining will concentrate on deposits around hydrothermal vents, and not on active vents or chimneys due to technical risks associated with high temperatures (Gwyther et al., 2008), it is likely that the background and extinct vent communities (from microorganisms to megafauna) will be impacted through habitat loss, mechanical destruction, noise, smothering and bioaccumulation of toxic substances (Levin et al., 2016). However, knowledge about the background ecosystem and natural plume is sparse, as the vents and their proximal fauna have attracted most of the attention, for example in microbiology (e.g. Han et al., 2018; Cerqueira et al., 2018).

To fill this gap, the Dutch TREASURE project (STW-NWO) was focussed on describing the structure of the background pelagic and benthic communities of an active hydrothermal vent site with SMS deposits on the Mid-Atlantic Ridge (MAR). The Rainbow hydrothermal vent ~~south of the Azores~~(36°14" N on the MAR) was selected for this study as it ejects one of the most prominent and persistent natural plumes on the MAR. Hydrothermal plumes represent a distinct natural ecosystem in itself, which under the influence of currents may extend tens of kilometres away from its point of origin. Basic knowledge of natural plumes is essential to be able to ~~assess~~ discern ~~mining~~ impacts arising from future SMS mining ~~consisting of for two reasons: firstly, hydrothermal plumes represent a distinct ecosystem in itself, which under the influence of currents may extend tens of kilometres away from its point of origin.~~ plumes created in the vicinity of the hydrothermal vent ~~during excavation and by discharge of the return flow~~ which are likely ~~may~~ interfere with the natural hydrothermal plume~~.~~ ~~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.~~ Though mining plumes will have a higher initial density and therefore tend to sink rather than maintain buoyancy (Gwyther et al., 2008; Boschen et al., 2013), the finest and slowest sinking fraction of suspended solids in the mining

plume may interfere with the natural plume during its dispersal, especially when released above the seafloor. ~~SecondlyFurther, understanding natural plume processes may reveal how ecosystems adapt to elevated turbidity and co-occurring changes in the chemical environment.~~

Since the discovery of the Rainbow hydrothermal vent field in 1996 by German et al., several ~~relationships~~ studies concerning the composition of the hydrothermal fluid and the ~~associated~~ sediment influenced by fall-out of particulates ~~formed by precipitation~~ from the Rainbow and other hydrothermal plumes have been ~~established~~published. These showed, ~~For~~ for example, ~~it was shown~~ that the underlying host rock influences the hydrothermal fluid composition (Wetzel and Shock, 2000; Marques et al., 2006). Geochemical investigation of sediment by Cave et al. (2002) at distances of 2 to 25 km from the Rainbow hydrothermal vent field 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. It has further been ~~demonstrated~~shown that microbial activity influences geochemical processes in the plume (Breier et al., 2012; Dick et al., 2013), such as scavenging and oxidation of metals (Cowen and Bruland, 1985; Cowen et al., 1990; Mandernack and Tebo, 1993; Dick et al., 2009). ~~Studies of other hydrothermal vent systems demonstrated that deposition from the plume is partially being influenced~~mediated by microbial activity ~~which enhances scavenging and oxidation rates of metals (e.g. Cowen and Bruland, 1985; Cowen et al., 1990; Mandernack and Tebo, 1993; Dick et al., 2009), with implications for~~ influencing the local ocean geochemistry.

Microbial activity within the plume is fuelled by redox reactions that provide energy for chemolithoautotrophic microbial taxa. The abundance of energy sources within plumes support a plethora of chemolithoautotrophic microbial communities (e.g. Orcutt et al., 2011; Frank et al., 2013; Anantharaman et al., 2016~~5~~). Plume microbial communities can be distinct or relatively similar to background communities (Dick and Tebo et al., 2010; Sheik et al., 2015; Olins et al., 2017), with plume associated bacteria originating from either seafloor communities, background seawater communities or from growth within the plume (Dick et al., 2013). Djurhuus et al. (2017) observed the ~~dilution~~reduction in dominance of vent associated microorganisms with increased redox potential, suggesting that communities associated with the initial rising plume ~~would~~ become diluted ~~disperse with distance from the vent~~ on a scale of metres ~~of metres, showcasing a variable community within the plume~~.

Comparatively little is known about changes in chemical composition and microbial assemblages in the hydrothermal plume after its initial rise, when it becomes neutrally buoyant and is dispersed -by currents, remaining traceable in particulate form to at least 50 km away from its source (Severmann et al., 2004), and even up to 4000 km is dissolved form (Resing et al., 2015). 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. ~~After its initial rise, a hydrothermal vent plume becomes neutrally buoyant and is dispersed in particulate form to at least 50 km away from its source (Severmann et al., 2015)over potentially hundreds of kilometres (German and Sparks, 1993; Dymond and Roth, 1988), and even up to 4000 km in dissolved form (Resing et al., 2015). , however t~~This ~~particulate portion of the plume, however, has not been sampled in a similar manner to identify microbial community patterns. Simulated experiments, howeverneverthless, suggest the majority of microbial growth occurs in the neutrally buoyant plume Reed et al., 2015).~~

~~Overall, little is known limited knowledge is present about the chemical fractionation or microbial assemblages within the neutrally buoyant plume as it ages and disperses from the hydrothermal vent field. 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. 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. However, the natural plume could serve as an analogue for the finest and slowest sinking fraction of suspended solids in the mining plume. In this study~~In order to address this gap, water column and sediment samples from the Rainbow hydrothermal vent area were investigated during the TREASURE cruise. Geochemical and biological changes were ~~tracked~~ exploredstudied 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. N~~and n~~ext generation sequencing methods were used to ~~study~~ quantify the microbial diversity ~~heterogeneity~~ in the ~~background~~ pelagic system that was influenced by the hydrothermal plume. 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 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. ~~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 whilst demonstrating potential monitoring tools to characterise these plumes.~~

2 Material and methods

2.1 Study site

The Rainbow hydrothermal vent field (Fig. 1) is located on the Mid Atlantic Ridge (MAR) at 36°13.80 N, 33°54.14 W at approximately 2300 m water depth, southwest of the Azores. The vent field is located on the western flank on the non-volcanic Rainbow Ridge, in an offset between the South Alvin Mid Atlantic Ridge (AMAR) and AMAR segments of the MAR (German et al., 1996; Fouquet et al., 1998; Douville et al., 2002). It is located at the intersection between the non-transform fault system and the ridge faults (Charlou et al., 2002), making this vent field tectonically controlled. 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; Marques et al., 2006). 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., 2002~~1997~~; Douville et al., 2002), resulting in relatively high metal/sulfide ratios. Consequently, the chimneys and the SMS deposits of the Rainbow hydrothermal field are enriched in Cu, Zn, Co and Ni when compared to vent systems with a basaltic host rock (Charlou et al., 2002~~1997~~).

The vent field consists of 10 active, high temperature (365 °C) black smokers and emits an extensive plume with a distinct chemical composition compared to the ambient seawater (Severmann et al., 2004). The plumeIt is considered the largest and widest spreading plume in the region (German et al., 1996), rising up to 200 m above its source and was traced traceable over at least 50 kilometres (Severmann et al., 2004). The plume dispersion is cControlled by the local hydrodynamic regime and topography (Thurnhnerr and Richards, 2001; Thurnherr et al., 2002), the neutrally buoyant plume movesing predominantly to the north and east around the Rainbow Ridge with an average current speed of 5-6 cm s^{-1} and continues in a northward direction along the southern and eastern side of the rift valley of the AMAR segments (Edmonds and German, 2004). Characteristics and behaviour of the Rainbow plume are relatively well-studied whichThe plume characteristics and prior knowledge of its behaviour make the Rainbow vent field a suitable site to study neutrally buoyant plumes.

2.2 Water column profiling and sediment sampling

Water samples and sediment cores were collected along the gradient path of the plume during RV *Pelagia* cruise 64PE398 in April 2015. Five putatively distinct biotopes were sampled: (i) above plume (1000 m water depth), (ii) plume, (iii) below plume (10 metres above bottom), (iv) near-bottom water and (v) sediment.

Using CTD casts with a Seabird 911 CTD-Rosette system, the plume was traced in real time using turbidity as an indicator, measured in NTU with a WETLabs turbidity sensor. Other variables measured included temperature (°C), salinity (PSU), density (σ-θ, $kg\ m^{-3}$), dissolved oxygen ($ml\ L^{-1}$) and chlorophyll ($\mu g\ L^{-1}$). At five stations, continuous yoyo CTD-casts were taken over the course of 12 hours, to study the temporal changes of the hydrothermal plume.

A total of 41- water samples were collected using 12 L Niskin bottles from eleven downstream stations, two distal downstream stations and three upstream stations. Once the CTD was back on deck, three distinct water samples were immediately taken for suspended particulate matter (SPM), trace metals, and

the microbial community. ~~Additional intermittent water samples were taken for nutrients and suspended particulate organic matter (Table 1).~~

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 mg L^{-1}. If possible, trace metal and microbial community samples were taken at the same stations and/or same depth.

Sediment and near-bottom water samples were collected with a NIOZ designed box corer of 50 cm diameter equipped with a top valve to prevent flushing, subsequently trapping ~~more than 1.5 litres of~~ near-bottom water (van Bleijswijk et al., 2015). In total eight cores were collected (Table 1). Due to unsuitable coring substrates, CTD locations and coring sites did not always follow the same track. Box ~~C~~cores were taken on the eastern part of the Rainbow Ridge, continuing in the basin east of the ridge, while two cores were taken on the north-western flank of the ridge, following the path of the plume.

2.3 Suspended particulate matter analysis

From each 12 L Niskin bottle, two 5 L subsamples were collected to determine the concentration of SPM. The subsamples were filtered on board over pre-weighed 0.4 µm polycarbonate filters. The filters were rinsed with ~10 ml of Milli-Q water to remove salt, while still applying under pressure, and subsequently stored at -20 °C on board. I~~Prior to analysis i~~n the laboratory, the filters were freeze dried ~~. The samples were~~and then weighed in duplo, or in triplo ~~once again~~ if the difference between the first two measurements was more than 0.03 mg ~~or more~~. 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). Subsequently, the filters were examined using a Hitachi TM3000 table-top scanning electron microscope (SEM) connected to an energy-dispersive spectroscopy (EDS)-detector to visualize content of the SPM and to qualitatively analyse the chemical composition. The SEM was operated under an acceleration voltage of 15 kV and a filament current of 1850 mA.

2.4 Chemical analysis

For analysis of major ~~In order to examine the~~and trace metals present in particulate form in and around the hydrothermal plume, water samples were filtered on board over acid-cleaned 0.45 µm polysulfone filters directly from the Niskin bottle at ambient temperature while applying under pressure. A water barrel in between the filtration holder and pump allowed for volume measurements of water filtered. The filters were subsequently stored at -20 °C until further examination. Filters ~~and~~ were ~~left to dry~~dried in the laboratory in an Interflow laminar flow bench at room temperature prior to analysis. Subsequently, ~~T~~the filters were placed in acid-cleaned Teflon vials and were subjected to a total digestion method. For this purpose a mixture of 6.5 ml HNO_3 (ultrapure)/HF (suprapure) (10:1) solution, 1 ml HCl (ultrapure) and 1 ml $HClO_4$ (ultrapure) was added to the vials, after which the vials were covered and placed in an Analab hotblock for 48 hours at 125 °C. After the filters were completely dissolved, the covers were taken off from the vials and the vials were left for 24 hours in order to evaporate the acids. Finally, the residue was taken up again in 10 ml 1M ultra grade HNO_3, pre-spiked with 5 ppb scandium and 5 ppb rhodium as internal standards. 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. These blanks were subjected to the same total digestion method as described above. A HR-ICP-MS (Thermo Element II) at the Royal Netherlands Institute for Sea Research (NIOZ) was used to analyse the concentrations of major- and trace metals, as well as REEs. 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. The machine drift was measured before, half-way and after each series of samples and was monitored by using an external drift solution. 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 %. The accuracy could not be determined as no certified reference material was analysed. 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.

2.5 Microbial community

Three distinct samples of 2 L of water were collected from three different Niskin bottles for Next Generation Sequencing (NGS). The water was filtered immediately after collection through a 0.2 µm polycarbonate filter (Nuclepore) facilitated by a vacuum of 0.2 bar, in a climate controlled room at 4 °C~~,~~ to limit DNA degradation. From the box cores~~With a sterilised spatula,~~ >0.25 grams of surface sediment were scraped off with a sterilised spatula, ~~from the box cores~~ whilst 1.5 litre of overlying (near-bottom) water was filtered as above. Filters were stored in a 2 ml cryo-vial and all samples were stored at -80 °C on board.

DNA was extracted using a Power Soil DNA Isolation Kit (MoBio, now Qiagen) according to the manufacturer's protocol. Each DNA extract concentration was quantified using a Qubit 3.0 fluorimeter (Qiagen, Inc.) and stored at -20 °C before amplification. Extracts were combined with Phusion Taq (Thermo Scientific), High Fidelity Phusion polymerase buffer and universal primers to amplify the V4 region of 16 S rDNA of bacteria and archaea (Table 2), with unique molecular identifier (MID) combinations to identify the different samples. All negative controls from all PCR series were labelled with the same unique MID. The PCR settings were as follows: 30s at 98 °C, 29 cycles (10s at 98 °C, 20s at 53 °C, 30s at 72 °C) and 7 minutes at 72 °C. Four and three samples were re-run at 30 and 32 cycles, respectively, in order to yield enough product. Each sample was subjected to the polymerase chain reaction (PCR) protocol in triplicate and processed independently to avoid bias. 5 µl of product was used to screen the products on an agarose gel. The remaining 25 µl of each triplicate was pooled to evenly distribute the DNA, split into two slots and run on a 2 % agarose gel at 75 volts for 50 minutes. Sybergold stain was applied post run for 20-30 minutes before cutting the 380 bp bands out with a sterilised scalpel over a blue light to avoid UV damage. The two bands of mixed triplicates were pooled, purified using the Qiaquick Gel Extraction Kit (Qiagen, Inc.) and quantified with a Qubit™ 3.0 fluorometer (Qiagen, Inc.).

Samples were pooled in equimolar quantities together with blank PCR controls. The pooled sample was concentrated using MinElute™ PCR Purification columns (Qiagen Inc.) as described by the manufacturer and sent to Macrogen (South Korea) for sequencing. Sequencing was undertaken with a Roches GS FLX instrument using Titanium chemistry on a one-eight region gasket and Roche GS FLX instruments. Sequence processing was undertaken as described by van Bleijswijk et al. (2015), using a QIIME pipeline. Sequences shorter than 250 bases and average Q scores below 25 were removed. The OTU sequences (>98 % similarity) were classified (>93 % similarity) based on a recent SILVA SSU database (release 132; Yilmaz et al. 2014). Single reads were excluded and all data were standardised to remove any disproportionate sampling bias.

2.6 Statistics

Unconstrained ordination techniques were utilised to distinguish biotopes and general community patterns. Non-metric Multi-Dimensional Scaling plots (NMDS) were created based upon Bray-Curtis similarity matrices of square root transformed microbial community assemblages. Group average clustering was also utilised in order to quantify similarities between the samples. ANalysis Of SIMilarities (ANOSIM) was subsequently used to statistically test community distinctions based upon presumed biotopes (sediment, near-bottom water, below plume water, plume water and above plume water). In addition, all water column samples were plotted in separate NMDS plots to observe~~d~~ patterns in greater detail. Physical properties of all water samples (station, depth, turbidity and location) were depicted in a NMDS plot to observe sample similarities. These environmental data were normalised and Euclidean distance was used to create a similarity matrix. The relationship between Fe and turbidity was tested with a linear regression analysis. Trace metals and REE were normalised to Fe, since it is the primary particle-forming element at all stages of plume dispersion, giving insight in the chemical behaviour. All multivariate statistics were undertaken in Primer™ V6 (Clarke and Gorley, 2006).

Shannon-Wiener index (log e) was calculated as a diversity measure. Biodiversity differences between biotopes were tested with the non-parametric test Kruskal-Wallis with pairwise comparisons as the data

did not meet normality or homogeneity assumptions, even after transformation. These statistical tests were undertaken in SPSS.

A SIMilarities PERcentage analysis (SIMPER in Primer v6) was applied on the microbial class level with a cut off for low contributions at 90 % based on Bray-Curtis similarity matrix to characterise the community composition based on groups contributing to intra biotope similarities. Relationships between environmental variables and microbial classes as a percentage of each composition within the plume, were tested with Pearson correlation and hierarchical clustering to identify broad response groups.

3 Results

3.1 Water column characteristics

Temperature, salinity and density plots indicated that the water column at each location had similar physical traits, whereby three main different water masses could be distinguished (Supplement Fig. S1). The surface Eastern North Atlantic Central Water (ENACW) was characterised by a temperature, salinity and density at the surface of 18 °C, 36.4 PSU and 26.2 kg m^{-3} to 11 °C, 35.5 PSU and 27.2 kg m^{-3} at the bottom of the water mass. The underlying Mediterranean Outflow Water (MOW) was characterised by a temperature of 7.5-11 °C, a salinity of 35.4-35.5 PSU and a density of 27.2-27.75 kg m^{-3}. The North Atlantic Deep Water (NADW) was characterised by temperatures ranging from 4 to 7.5 °C, salinity of 35.0 to 35.4 PSU and a density of 27.75 to 27.825 kg m^{-3} (Emery and Meincke, 1986). The neutrally buoyant plume was centred around the 27.82 kg m^{-3} isopycnal, as illustrated in Figures 2 and 3.

3.2 Turbidity and plume dispersion

Against a background of non-plume influenced waters, as found in the CTD casts, with typical concentrations of SPM of 0.04 mg L^{-1} (0.015 NTU), the neutrally buoyant plume stands out as a layer ~~of~~ with distinctly higher turbidity values (i.e. higher SPM concentrations) consistently present in the depth interval of 1750 – 2400 m at stations located north and east of Rainbow (Fig. 2). Except where this turbid

water layer was found impinging the seabed, relatively clear waters separated the turbid layer from the underlying seabed. ~~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.~~

At downstream stations, a consistent trend of decreasing turbidity and increasing vertical dispersion was noted. At station 27, 3.5 km north of Rainbow, maximum turbidity in the core of the plume was 0.15 NTU (0.09 mg L^{-1}) and plume thickness was about 105 m, whilst at station 46, 15.2 km east of Rainbow, maximum turbidity was only 0.08 NTU (0.06 mg L^{-1}) and plume thickness was 275 m. Away from the main plume path, station 47 and 49 (13.8 and 16.5 km from Rainbow, respectively) showed a diluted signature similar to that observed at the most distal stations along the main plume path. Despite being most proximal to Rainbow, station 16, located 1.0 km downstream of Rainbow, showed a relative low turbidity of 0.015 NTU (0.04 mg L^{-1}). Since the plume is more constrained closer to the source, the main body of the narrower plume could have been missed with the CTD. Stations upstream of the vent site (station 13 and 28, 4.2 and 7.5 km southwest of Rainbow respectively and station 40, 3.6 southeast of Rainbow) displayed low turbidity values, ranging between 0.01 and 0.02 NTU (0.04 mg L^{-1}) (Fig. S2).

The CTD profiles from stations 42 and 49 (4.9 and 16.5 km north of Rainbow respectively) both displayed highest turbidity in the lower hundreds of metres above the seafloor, with instances of seafloor contact during time of sampling. Therefore no samples could be taken below the plume at these stations. The assumption that the plume is subject to vertical movement is supported by observations made during 12-hour CTD yoyo casts carried out at station 27 (Fig. 3). Along with vertical displacements of the 27.82 kg m^{-3} isopycnal on the order of 150 m, likely reflecting internal tidal motions, the hydrothermal plume was found to also move up and down, at times touching the seafloor.

3.3 Enrichment of (trace) metals compared to the ambient seawater

NMDS ordination (Fig. 4) based on Euclidean distance resemblance of normalised element/Fe molar ratio data of all collected water samples (2D stress = 0.03), revealed a clear distinction of the different samples.

Most outstanding are the samples from above plume waters, indicating that the chemical composition is different from the other samples.

The remaining samples showed less variation, nonetheless the samples collected from below the plume and the samples collected away from the main path of the plume can be distinguished. This shows that the hydrothermal plume can be characterised by its chemical composition. When comparing samples taken in the turbidity maximum of the plume to the above plume water samples taken at 1000 m water depth it is found that Fe, Cu, P, V and Pb are enriched by factors of ~80, ~90, ~17, ~52 and ~25 respectively. Elements with a more moderate degree of enrichment are Co, Mn, Zn, Al and Ni, with enrichment factors of ~8.0, ~2.5, ~10.3, ~1.4 and ~1.6, respectively. The REEs were enriched by a factor of 5 to 40 relative to the clear water. U, Ti and Ca are slightly enriched at turbidity maxima, by factors of ~1.3, ~1.6 and ~1.2, respectively. In and Sn are depleted compared to the above plume water~~clear water above the plume~~.

3.4 Geochemical gradients within the hydrothermal plume

Within the hydrothermal plume, geochemical evolution is found as the plume disperses. Visual examination of the samples with the SEM coupled with chemical analysis performed with the EDS-detector revealed that the SPM within the plume close to the Rainbow hydrothermal vent at station 32 (2.9 km north of Rainbow) mainly consisted of Fe-sulfides. In the plume samples further downstream, Fe is mainly present as Fe-oxides, Fe-hydroxides or bound in alumino-silicates.

Chemical examination of the samples showed gradients in the element/Fe molar ratios along the path of the plume as well as off the main path of the plume at upstream and the most distal downstream stations. Since the Fe concentration is linearly related to the turbidity (Fig. 5) (R^2 = 0.9356, $P < 2.2*10^{-16}$), normalisation to Fe reveals relative enrichments or depletion of common elements. The chalcophile elements Co, Cu and Zn show a partly-linear relation steepening with increasing Fe concentration (Fig. 6A for Cu), indicating that the element/Fe molar ratios are elevated close to the source but decrease towards the more distal sites (Fig. 7A). One exception is the Zn/Fe molar ratio, which is elevated at station

37, 39 and 44. Furthermore, a high Zn/Fe molar ratio is observed at upstream station 40. The oxyanions P and V are linearly related to Fe (Fig. 6B for V), ~~therefore they also display more or less constant molar ratios,~~ and shows~~s~~ varying element/Fe molar ratios without a clear trend of increasing or decreasing ratios, both upstream and downstream of Rainbow (Fig. 7B). The REE show a partly-linear relation levelling-off with increasing iron concentrations (Fig. 6C for Y). Within the plume this is displayed as increasing element/Fe molar ratios towards station 44, with station 42 as an exception, followed by ~~a constant or~~ slightly decreasing molar ratios from station 44 onwards (Fig. 7C). The Ca/Fe molar ratios ranged between 0 and 15 for most of the downstream stations, apart from the stations further downstream (47 and 49), which displayed slightly higher Ca/Fe molar ratios. Upstream station 28 had a Ca/Fe molar ratio similar to those found at station 47 and 49 and upstream station 40 was found to have a significantly higher Ca/Fe molar ratio (Fig. 7E). Other analysed elements, Mn, Al, Ni, In, Pb, Sn, Ti and U showed no clear relationship with the Fe concentration (Fig. 6D for Sn). However, within the plume it was found that the Mn/Fe molar ratio is lower than at the upstream stations or the more distal downstream stations.

3.5 Microbial assemblages in water column biotopes

Samples from sediment, near-bottom water and ~~above~~ no plume water contained microbial communities which clustered distinctly from each other and from plume, ~~and~~ below-plume and above-plume communities (Fig. 8). In particular, sediment, ~~and~~ near-bottom water and no~~n~~-plume (station 13) samples have communities that are very dissimilar from the overlying water column samples. Sediment samples appeared to cluster in a straight line suggesting some sort of gradient of similarity along the ordination axis, though no apparent patterns were observed when independently plotted. The near-bottom water samples were relatively dispersed in the NMDS plot suggesting a more variable community. Samples taken at the upstream station 13 from below-plume and plume depths showed no similarity with ~~analogous~~ samples from corresponding depths ~~from~~in the other stations, ~~except for~~whilst the ~~above~~ above-plume community at this station ~~which~~ is consistent with that of other stations. In general, plume and below-plume communities were more similar nearer to the vent source, with stations further downstream displaying greater dissimilarity (Fig. 9, Fig. S3).

Group average cluster analysis showed high level of dissimilarity, i.e. large community variation, between and within biotopes. ANOSIM revealed all putative biotopes that were sampled had distinct communities (Global R = 0.738; p = 0.001; 999 permutations), except for plume and below plume samples which could not be distinguished statistically (Global R = -0.091; P = 0.861). The two seemingly unique samples from station 13 also tested significantly distinct, but with a low number of permutations (<999) due to low replication (n=2).

3.6 Univariate biodiversity

Plume and below plume samples were less diverse than sediment samples, whilst diversity in the plume was lower than in near-bottom water samples (Kruskal-Wallis: $\chi 2$ (4) = 36.127, P < 0.01). In general, plume diversity was low (Fig. 10), but further differences were not statistically significant, likely due to limited replication and intra biotope variation.

The plume microbial community at sites upstream of Rainbow and at the immediate downstream sites (stations 28, 16 and 27) showed similar and relatively high biodiversity (>4.5) (Fig 11). Plume biodiversity at the sites further away from Rainbow gradually decreased until station 46, which displayed the lowest Shannon-Wiener index value of 2.4. Distant stations 47 and 49, showed biodiversity rising to a more moderate index value around 3.5.

3.7 Species composition

Results of the SIMPER analyses showing the contributions of taxa composition to similarities within biotopes (Table 3), mirrored the NMDS and ANOSIM results whereby the similarity of community composition in each biotope was dominated by a different makeup of the microbial community. The Archaeal class Nitrososphaeria (Marine group 1 archaea) contributed the most to similarity within the above and below plume water communities, while also being very common in all water samples. Alphaproteobacteria, Gammaproteobacteria and Deltaproteobacteria also constituted as a large makeup

of all biotopes in the area. The class Epsilonproteobacteria were largely absent from above plume samples being not influenced by the plume, and only contributed <2 % to near-bottom water communities. By contrast, Epsilonproteobacteria were dominant in plume water samples (accounting for >35 % of the community), and were the fifth most dominant taxon in below plume water samples contributing 8.9 % of the community.

Epsilonproteobacteria accounted for about 20 % of the plume community at stations near the vent. Beyond the near vent stations, an increase in relative abundance of Epsilonproteobacteria with distance from vent was observed, accounting for 64 % of the community at the distant station 46 (Fig. 12). Alphaproteobacteria, Deltaproteobacteria and Gammaproteobacteria appeared to become less dominant with distance from the plume source (Fig. 12). The communities at distant stations 47 and 49 were less dominated by Epsilonproteobacteria (around 40 %). Below plume communities were dominated mostly by Nitrososphaeria (Marine group 1 Archaea) whereby Nitrosphaeria became more dominant with distance from the plume source likewise as the Epsilonproteobacteria in the plume. Correlations between environmental variables (elemental chemistry and physical properties) and all microbial classes observed in the plume were evident and appeared class specific (Fig. S4). The hierarchical clustering revealed eight broad response groups, which displayed different relationships with the environmental variables.

4 Discussion

Using a multidisciplinary approach in which physical, geochemical and ecological data were collected from the Rainbow vent neutrally buoyant plume and its underlying sediment, we aimed to expand knowledge and characteristics of the ~~T0~~ background (i.e. before impact) state of ~~the background ecosystem of~~ a hydrothermal vent. Such knowledge is deemed essential to be able to assess (potential) impacts of future deep-sea SMS mining, as it may help in characterising the behaviour of the dilute distal part of chemically enriched mining plumes. We found geochemical and microbial differences between the above-plume, plume, below plume and no-plume water- and ~~and background water composition with identified distinct biotopes. I~~in addition, pertinent chemical and biological gradients within the extensive Rainbow hydrothermal vent plume were evident.

4.1 Physical constraints of plume location and behaviour

The plume was observed within the NADW mass, constrained to an isopycnal density envelope of 27.82 kg m^{-3} (Fig. 2 and 3). 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 local sediment resuspension as origin. 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 ~~in agreement with observations made~~ by German et al. (1998) who found that the Rainbow ~~of a~~ plume extends over ~~greater than~~ 50 km, being ~~that is~~ controlled by local hydrodynamics and topography. Unexpectedly, in the basin upstream of the Rainbow vent field a turbidity peak at 1975 m water depth resembling a plume was observed as well (station 28), confounding our assumption of a clear water column at upstream stations and distant downstream stations. This ~~indicates~~ suggests that the plume is ~~reaching~~ distributed much further than previously observed by Thurnherr and Richards (2001) and German et al. (1998). This is exemplified by the local variation in microbial community composition of upstream stations (Fig. 12) and is supported by the relatively low Ca/Fe molar ratio at station 28 (Fig. 7), indicating hydrothermal influence. In addition, the observed variability of plume strength and vertical position (Fig. 3) indicate that local fluctuation in the current regime and tidal motions influence the plumes behaviour. This dynamic behaviour has implications for surveys designs and should be considered when monitoring natural and man-made plumes, such as mining-related plumes. Prior insight into plume extension and behaviour is required for the identification of adequate control sites and for tracking of plume evolution in future impact studies.

4.2 Plumes influence on the water column chemical and microbial make-up

The neutrally buoyant plume introduced pelagic heterogeneity in terms of chemical and microbial composition, which is supported by the vertical classification of the different biotopes. The neutrally buoyant plume was evidently enriched in metals and REE compared to overlying clear water. Element

concentrations were found to be in line with those found by German et al. (1991) and Edmonds and German (2004) who have studied the Trans-Atlantic Geotraverse (TAG) hydrothermal plume and the Rainbow hydrothermal plume, respectively. Our chemical results from Rainbow also match with those of Ludford et al. (19966~~5~~), who have studied vent fluid samples from the 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).

The distinctive chemical composition of the plume samples (e.g. metal concentrations) affects chemolithoautotrophic microbial growth within the plume as indicated by the typical microbial community in plume samples. Unlike Sheik et al. (2015), we observed a clear and consistent separation between communities in the plume and those in above-plume samples. The influence of MOW on the above-plume community could also play a role, as ~~oceanic~~ water masses can harbour different microbial communities (Agogue et al., 2011). However, the palpable presence of a plume in the turbidity data with supporting chemical measurements, and the occurrence of vent associated Epsilonproteobacteria (Olins et al., 2017; Djurhuus et al., 2017) and other vent associated groups such as the Gammaproteobacteria clade SUP05 (Sunamura et al., 2004), point to a unique chemical environment. Here chemosynthetic communities flourish and give rise to independent biotopes in the neutrally buoyant plume kilometres downstream of the vent site.

Below-plume communities were not distinct from the plume biotope, although instead of Epsilonproteobacteria, the ubiquitous class Nitrososphaeria was the most dominant group, reflecting some similarities with above-plume seawater communities. Similarities between plume and proximal habitat communities haves ~~s~~ also been observed by Olins et al. (2017), whereby intra-field (defined as within vent field between diffuse flows) and diffuse flow microbial communities were alike. In our study, similarities between plume and below-plume are likely derived by precipitation of mineral and microbial aggregates dragging plume microbes deeper below the plume as suggested by Dick et al. (2013). In addition, internal wave induced turbulence causes vertical mixing along the slope of the Rainbow Ridge (van Haren et al., 2017), which may cause the plume and associated communities near the vent field to mix with ambient water communities leading to assemblage similarities. This indicates the plume and

associated microbial processes could have a larger vertical footprint than previously observed, supporting suggestions by Olins et al., (2017) that proximal non-plume habitats have been overlooked. Interestingly, near-bottom water (and sediment) community assemblages were distinct from the below-plume and other water column communities. This could imply: 1) that there is little ”fall out” from the plume at distance from the vent which is in agreement with sediment trap observations by Khripounoff et al. (2001), 2) plume specific bacteria die off due to lack of energy sources and DNA degrades before reaching the seafloor, 3) microbes are more abundant in the near-bottom waters, either naturally or through mechanical disturbance resuspending sediment during the coring process, outnumbering groups that have been mixed in from overlaying water. Despite the presence of a plume and precipitation, a barrier between the sea floor and the water column biotopes is present, consistent with global broad scale non-vent benthic-pelagic patterns (Zinger et al., 2011). According to Khripounoff et al. (2001) particulate~~the~~ fall-out from the Rainbow plume is spatially very limited. This implies that~~, as~~ the extended chemical imprint on the sediment (reported by Cave et al. (2002), Chavagnac et al. (2005), and this study), is likely to have formed when the plume is in direct contact with the sediment during its vertical tidal migration. As the plume rises again, the associated distinct communities apparently resume dominance in the near-bottom water. Though Epsilonproteobacteria have been detected in Rainbow vent sediments comprising over 5 % of the sediment community (Lopez-Garcia et al., 2003), very few reads of this group in sediment samples were present in our study probably as our coring samples were collected km’s away from the venting site. Cave et al. (2002), observed chemical evolution of sediment composition with distance from source, thus we infer ~~the dependence~~a relationship between the ~~of~~ sediment dwelling Epsilonproteobacteria ~~on~~ with nearby plume precipitates, such as Cu ~~-~~and presumed precipitates~~,~~ Zn and Cd (Trocine and Trefry, 1988). Additionally, DNA degradation rate can be 7 to 100 times higher in sediment than in the water column (Dell’Anno and Corinaldesi, 2004). Therefore, although our results suggest no microbial plume community imprint on the sediment, we cannot rule out short lived episodic community changes when the plume is in contact with the sediment.

4.3 Geochemical gradients within the hydrothermal plume

Analysis of SPM in water samples taken along the flow path of the plume, as well as off the flow path, showed conspicuous trends of elements, reflecting the chemical evolution of the plume as it drifts away from its hydrothermal source.

The chalcophile elements (Cu, Co and Zn) were found to have the highest element/Fe molar ratios closest to the vent site, indicating either rapid removal from the hydrothermal plume or removal from the solid phase as the plume drifts away from the vent site. Using SEM-EDS, it was demonstrated that at the proximal downstream stations mainly Fe-sulfides were found, whereas Fe-(oxyhydr)oxides were found further downstream. This suggests that chalcophile elements are mainly present in the form of sulfide mineral particles at the proximal stations, which are entrained in the flow of hydrothermal water emanating from the Rainbow vents and subsequently rapidly lost by settling from the plume in sulfide-bearing phases, while a large portion of Fe remains is suspension (Cave et al., 2002; Edmonds and German, 2004), consistent with decreasing concentrations of Cu, Zn and Co~~r~~ in sediment recovered from the Rainbow area with increasing distance to the vent site (Cave et al., 2002).

The oxyanions (V and P) showed ~~constant~~ slightly varying element/Fe molar ratios with increasing distance away from Rainbow, suggesting co-precipitation with Fe as oxyhydroxides (Edmonds and German, 2004). No additional uptake of these elements was observed with increasing distance from the vent field (German et al., 1991), since these elements are scavenged initially in significant amounts during the buoyant plume phase (Cave et al., 2002).

The trend shown by Mn/Fe molar ratios can be attributed to the slower oxidation kinetics of Mn (Cave et al., 2002). It takes longer for reduced Mn to be oxidised than it would for Fe, resulting in an increase in particulate Mn with increasing distance from the Rainbow hydrothermal vent field, which subsequently settles out from the plume as Mn-oxyhydroxides (Cave et al., 2002).

The observed positive relationship between the REEs and Fe is indicative of continuous scavenging of these elements from the ambient seawater onto Fe-oxyhydroxides (Edmonds and German, 2004; Chavagnac et al., 2005; Caetano et al., 2013). Therefore, the highest element/Fe molar ratios were observed away from the Rainbow hydrothermal vent site, where Fe-(oxyhydr)oxides are dominant more distal to the vent site.

The Ca/Fe molar ratios vary between 0 and 154 for the stations downstream of the Rainbow hydrothermal vent, but are higher at the distant downstream station 47 and 49 and upstream stations 28 and 40. Especially at station 40, located on the Rainbow Ridge, the Ca/Fe molar ratio is significantly higher than at the other stations. This is in line with observations by Khripounoff et al. (2001) and Cave et al. (2002) who also found that the relative Ca concentration in settling particles and the sediments is lower close the Rainbow vent field and increases as the Fe concentration decreases when the plume disperses. Since Ca is naturally present in high abundances in pelagic skeletal carbonate which rains down from the overlying water column and Fe is mainly present as a hydrothermal component the Ca/Fe molar ratio could be an indicator for the extent of the hydrothermal influence. The high molar ratio at station 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 stations directly downstream of Rainbow.

4.4 Microbial gradients within the hydrothermal plume

The microbial plume community composition and diversity altered with distance from the plume source showcasing a horizontal heterogeneity within the plume. Despite dilution, the vent associated group Epsilonproteobacteria (specifically the most common genus *Sulfurimonas*), appeared to dominate the community composition. This is likely due to its flexibility to exploit mainly sulfur compounds as electron donors, and oxygen and nitrate as acceptors (Nakagawa et al., 2005), making them suitable inhabitants of dynamic environments (Huber et al., 2003). From the relative abundance data presented here it cannot be determined ~~It is unclear from the relative abundance data obtained,~~ whether Epsilonproteobacteria dominate by rapid reproduction or if other groups decline in abundance. However, it is evident that Epsilonproteobacteria remain competitive or outcompete other competitors such as generalists Gammaproteobacteria that are often vent associated (i.e. SUP05). It is unlikely that this pattern is caused by entrainment of Epsilonproteobacteria from background seawater over time. This is based on the lack of significant presence of Epsilonproteobacteria in above-plume water and at remote station 13, and

reduced mixing that neutrally buoyant plumes generally experience (McCollom, 2000). This is further supported by the increasing uniqueness of the plume community with distance from the source, suggesting that mixing and entrainment between downstream biotopes is negligible.

The neutrally buoyant plume is likely too chemically enriched for non-adapted microbial taxa to thrive, and consequently are outcompeted by groups that can benefit from or tolerate the chemical nature of the plume. Therefore, it is likely that less specialised groups die out due to lack of appropriate resources and interspecies competition, as indicated by the decline in biodiversity with age of plume (distance) directly mirroring the increasing dominance of Epsilonproteobacteria, a group already known to influence diversity and community structures (Opatkiewicz et al., 2009; Sylvan et al., 2012). In addition, the decrease in concentration of particulate matter may influence microbial diversity (Huber et al., 2003). Temporal succession has been observed within plume environments by Sylvan et al., 2012 and Reed et al., 2015, driven by metabolic energy yield and concentration of the electron donors. ~~We propose that the patterns in our study~~These patterns may relate to ~~reflect~~ ecological succession (Connell and Slaytor, 1977) within the plume with change in microbial communities resulting in a low diversity, climax plume community. At the distant stations 47 and 49, the community was less dominated by Epsilonproteobacteria and more diverse, indicating a gradual return to what is ~~likely~~ possibly a non-plume influenced state of the microbial community. The wide range of correlations within and between microbial classes and water properties, i.e. ranging from chemical to physical variables (Fig. S4), indicates a complex array of community drivers within the plume.

In contrast to our results, Sheik et al. (2015) and Djurhuus et al. (2017), observed decreasing Epsilonproteobacteria abundance within hundreds of metres from the source in the rising, buoyant portion of plumes generated by Indian Ocean and South Pacific vents. Interestingly, in our results Epsilonproteobacteria were least dominant in the ~~freshest~~ neutrally buoyant plume~~fluid~~ ~~at the station~~ closest to the Rainbow vent site~~.~~, which may indicate~~Due to short lived rising plume relative to chemolithoautotrophic doubling time (Reed et al., 2015), It is likely~~ that entrainment of other microbial groups within the rising portion of the plume initially dilutes the contribution of Epsilonproteobacteria~~by this group~~., whilst the competitive advantage of this group becomes only evident at a later stage as the

plume drifts away from the source. However, Huber et al., 2003 suggested that Epsilonproteobacteria, thrive in weaker diffuse flow due to lower temperature and great electron acceptor availability, suggesting greater habitat suitability away from the immediate venting orifice. Furthermore, it has been demonstrated that Epsilonproteobacteria (specifically *Sulfurimonas*) have higher dispersal capabilities than thermophilic vent associated microbial groups (Mino et al., 2017). A sampling design to follow the continuity of the plume from the buoyant to the neutrally buoyant portion would be a suitable approach to fully trace the evolution of the plume from the orifice to full dilution. However, the term full dilution is ambiguous as it is unknown exactly how far the plume influences the water properties and how far the plume associated bacteria will follow, adding water column microbial community heterogeneity beyond our study spatial extent.

4.5 Possible effects of SMS mining plumes

Mining of SMS deposits will create additional plumes generated by activities of mining vehicles (resuspension) and by the discharge of solids from the surface vessel (discharge plume). It is yet unknown how these plumes will affect the ecosystem at active and inactive hydrothermal vent sites. Our study showed the influence of a natural hydrothermal plume on ~~its environment up to 25 km away from its source and it was shown how a natural plume has a strong impact on~~ the pelagic microbial and chemical composition up to 25 km away from its source. Not unlikely, the dispersion of sediment and chemically reactive mineral material in the water column may cause similar or larger changes to the background state. ~~, suggesting that mining plumes may cause similar changes to the background T0 state.~~

~~Excavation of SMS will cause removal of habitat by substrate extraction and resuspension of surface sediments.~~ While large particles mobilised by mining ~~in the resulting plume~~ are expected to stay close to the seafloor and ~~eventually~~ settle out rapidly, smothering fauna in the immediate surroundings (Jones et al., 2018), smaller particles will disperse further, potentially invoking effects on a larger spatial scale. ~~Another main concern is the discharge of mining waste, consisting of very fine unconsolidated particles, toxic metals and metal compounds (Weaver et al., 2018).~~ Modelling the behaviour of the discharge plume generated by the proposed Solwara 1 SMS mining has shown that these plumes can extend up to 10 km

from the mining site, resulting in a deposit thickness of up to 50 cm within 1 km of the discharge site (Gwyther et al., 2008; Boschen et al., 2013)., smothering benthic fauna (Boschen et al., 2013; Weaver et al., 2018; Jones et al., 2018). Apart from the physical impact that suspended fine-grained solids may have especially on suspension feeders, the presence of chemically reactive material may give the mining plume a distinct chemical and microbial fingerprint, analogues to a certain context to what we observed in the natural plume.Besides the impact caused by settling of particles from the excavation and discharge plumes, there is also the possible input of nutrients and toxic metals toxins to otherwise nutrient- and toxin-poor systems, for example from oxidation of newly exposed sulfides and the subsequent release of heavy metals in the water column (Jones et al., 2018; Weaver et al., 2018).

The extent of the local impact of deep deep-sea mining will depend on the location where the mining takes place. At an active site like the Rainbow hydrothermal vent field, we showed that even in the distant plume (25 km away from Rainbow) hydrothermal plume microbiota dominate. When a mining discharge plume at an active hydrothermal vent field would be merged with the natural plume, the local effects might be minimal since microbial communities are already adapted to the metal-rich environments (Gwyther et al., 2008). However, a mining plume consisting of a dense suspension of bottom sediment and fine-grained metal sulfides is expected to support an altered microbial community in terms of abundance and composition, impacting the hydrothermal plume community. Moreover, the effects over larger spatial scales could be multiplied because of the increased export of electron donors by mining activities. Reed et al. (2015), who studied a hydrothermal plume in the Lau basin, have shown that the export of the chemolithoautotrophs from a plume increases with increasing availability of electron donors. Dispersion of chemolithoautotrophs is variable between groups depending on the energetics of their metabolisms, for example, methanotrophs which could disperse more than 50 km, are likely to disperse further than sulfur oxidisers (Reed et al., 2015). Increased export of microbial biomass from plumes may have impact on other marine systems which are hospitable to chemolithoautotrophs, such as oxygen minimum zones (Dick et al., 2013) and to higher trophic levels (Phillips, 2017). At inactive sites the effect on the background fauna is also potentially large since these are not adapted to the heavy metal rich environments and the discharge plume could prove to be toxic to the fauna (Boschen et al., 2013), possibly affecting organisms at all levels of the food chain (Weaver et al., 2018). In addition, in case of multiple

plumes at different depths due to stratification and vertical migration due to tidal regimes, the impacts may not be confined to a single depth band and may affect a large part of the water column, including other habitats, such as benthic habitats.

5 Conclusion

Our results demonstrate geochemically enriched plumes provide a dynamic habitat that is conducive to ecological changes in a short time span. Combining microbial and chemical analysis has proven to be a sensitive tool which enabled us to trace the hydrothermal plume up to~~beyond~~ 25 km downstream from the vent source and also upstream of the Rainbow vent site, implying that the influence of the hydrothermal vent on the surrounding environment may reach further than previously thought. The neutrally buoyant plume was chemically enriched which spawned a distinct microbial biotope ~~which was~~ dominated by vent associated species. As the plume aged and dispersed we observed alteration of the chemical composition and microbial community composition of the plume, showcasing a horizontal heterogeneous plume. Overall we have shown that a hydrothermal plume acts as a unique chemically enriched environment where distinct and variable microbial habitats are present. The plume heterogeneity and its dynamical behaviour would require extensive sampling in order to be able to assess the impacts and interferences by man-made mining plumes on the natural conditions.

Data availability

CTD data presented in this work, filter weights for SPM sampling, geochemical data of the (trace) metals and REE, associated calculated enrichment factors and information on the blanks, drift measurements and detection limits of the HR-ICP MS analyses will be submitted to PANGAEA when the paper is published and are also available in the NIOZ data portal (https://dataverse.nioz.nl/dataverse/doi under DOI 10.25850/nioz/7b.b.s).

Raw sequence data will be available via the European Nucleotide Archive (ENA) under accession number PRJEBXXXXX, once the paper is published.

Author contribution

GD, HDS, and FM conceptualised the study and undertook data collection. SH and DP undertook sample processing and analysis with contributions from and under the supervision of FM, GD, GJR, HDS, JvB and HW. SH and DP wrote the manuscript with contributions from all co-authors.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

This study was carried out in the framework of the TREASURE (Towards Responsible ExtrAction of SUbmarine REsources) project, funded (grant number 13273) by the Applied and Engineering Sciences (AES) domain of the Netherlands Organisation for Scientific Research (NWO) and by partners from the Dutch maritime industry. Topsector Water, a collaborative effort of Dutch industry, academia and government, funded ship time. We thank Evaline van Weerlee for assistance in DNA extraction and Patrick Laan for assistance in the chemical analysis of the collected samples. We also thank the crew and captain of the RV *Pelagia*, as well as NIOZ technicians for their essential assistance during cruise 64PE398. SH received funding from the Blue Nodules project, EC grant agreement. 688785. DP is supported by the Natural Environmental Research Council [grant number NE/N012070/1]. HdS received funding from TREASURE. FM is supported financially by the Innovational Research Incentives Scheme of the Netherlands Organisation for Scientific Research (NWO-VIDI grant 016.161.360).

References

Agogue, H., Lamy, D., Neal, P. R., Sogin, M. L., and Herndl, G. J.: Water mass-specificity of bacterial communities in the North Atlantic revealed by massively parallel sequencing, Mol Ecol, 20, 258-274, https://doi.org/10.1111/j.1365-294X.2010.04932.x ~~10.1111/j.1365-294X.2010.04932.x~~, 2011.

Anantharaman, K., Breier, J. A., and Dick, G. J.: Metagenomic resolution of microbial functions in deep-sea hydrothermal plumes across the Eastern Lau Spreading Center, Isme J, 10, 225-239, https://doi.org/10.1038/ismej.2015.81, 2016.

Boschen, R. E., Rowden, A. A., Clark, M. R., and Gardner, J. P. A.: Mining of deep-sea seafloor massive sulfides: A review of the deposits, their benthic communities, impacts from mining, regulatory frameworks and management strategies, Ocean Coast Manage, 84, 54-67, https://doi.org/10.1016/j.ocecoaman.2013.07.005, 2013.

Breier, J. A., Toner, B. M., Fakra, S. C., Marcus, M. A., White, S. N., Thurnherr, A. M., & German, C. R.: Sulfur, sulfides, oxides and organic matter aggregated in submarine hydrothermal plumes at 9 50′ N East Pacific Rise. Geochim. Cosmochim. Acta, 88, 216-236, https://doi.org/10.1016/j.gca.2012.04.003, 2012.

Caetano, M., Vale, C., Anes, B., Raimundo, J., Drago, T., Schimdt, S., Nogueira, M., Oliveira, A., and Prego, R.: The Condor seamount at Mid-Atlantic Ridge as a supplementary source of trace and rare earth elements to the sediments, Deep-Sea Res Pt Ii, 98, 24-37, https://doi.org/10.1016/j.dsr2.2013.01.009, 2013.

Cave, R. R., German, C. R., Thomson, J., and Nesbitt, R. W.: Fluxes to sediments underlying the Rainbow hydrothermal plume at 36 degrees 14-' -N on the Mid-Atlantic Ridge, Geochim Cosmochim Ac, 66, 1905-1923, https://doi.org/10.1016/S0016-7037(02)00823-2, 2002.

Cerqueira, T., Barroso, C., Froufe, H., Egas, C., and Bettencourt, R.: Metagenomic Signatures of Microbial Communities in Deep-Sea Hydrothermal Sediments of Azores Vent Fields, Microb Ecol, 76, 387-403, https://doi.org/10.1007/s00248-018-1144-x, 2018.

Charlou, J. L., Donval, J. P., Fouquet, Y., Jean-Baptiste, P., and Holm, N.: Geochemistry of high H_2 ~~(2)~~ and CH_4 ~~(4)~~ vent fluids issuing from ultramafic rocks at the Rainbow hydrothermal field (36 degrees 14-' N, MAR), Chem Geol, 191, 345-359, https://doi.org/10.1016/S0009-2541(02)00134-1, 2002.

Chavagnac, V., German, C. R., Milton, J. A., and Palmer, M. R.: Sources of REE in sediment cores from the Rainbow vent site (36 degrees 14–' N, MAR), Chem Geol, 216, 329-352, https://doi.org/10.1016/S0009-2541(02)00134-1, 2005.

Clarke, K. R., Gorley, R. N.: PRIMER v6: User Manual/Tutorial (Plymouth Routines in Multivariate Ecological Research), PRIMER-E, Plymouth, 2006

Collins, P. C., Croot, P., Carlsson, J., Colaço, A., Grehan, A., Hyeong, K., Kennedy, R., Mohn, C., Smith, S., and Yamamoto, H.: A primer for the Environmental Impact Assessment of mining at seafloor massive sulfide deposits, Mar Policy, 42, 198-209, https://doi.org/10.1016/j.marpol.2013.01.020, 2013.

Connell, J. H., and Slayttyer, R. O.: Mechanisms of Succession in Natural Communities and Their Role in Community Stability and Organization, Am Nat, 111, 1119-1144, https://doi.org/10.1086/283241, 1977.

Cowen, J. P., and Bruland, K. W.: Metal Deposits Associated with Bacteria - Implications for Fe and Mn Marine Biogeochemistry, Deep-Sea Res, 32, 253-&, https://doi.org/10.1016/0198-0149(85)90078-0, 1985.

Cowen, J. P., Massoth, G. J., and Feely, R. A.: Scavenging Rates of Dissolved Manganese in a Hydrothermal Vent Plume, Deep-Sea Res, 37, 1619-1637, https://doi.org/10.1016/0198-0149(90)90065-4, 1990.

Dell'Anno, A., and Corinaldesi, C.: Degradation and turnover of extracellular DNA in marine sediments: Ecological and methodological considerations, Appl Environ Microb, 70, 4384-4386, https://doi.org/10.1128/AEM.70.7.4384–4386.2004, 2004.

Dick, G. J., Clement, B. G., Webb, S. M., Fodrie, F. J., Bargar, J. R., and Tebo, B. M.: Enzymatic microbial Mn(II) oxidation and Mn biooxide production in the Guaymas Basin deep-sea hydrothermal plume, Geochim Cosmochim Ac, 73, 6517-6530, https://doi.org/10.1016/j.gca.2009.07.039, 2009.

Dick, G. J., and Tebo, B. M.: Microbial diversity and biogeochemistry of the Guaymas Basin deep-sea hydrothermal plume, Environ Microbiol, 12, 1334-1347, https://doi.org/10.1111/j.1462-2920.2010.02177.x, 2010.

Dick, G. J., Anantharaman, K., Baker, B. J., Li, M., Reed, D. C., and Sheik, C. S.: The microbiology of deep-sea hydrothermal vent plumes: ecological and biogeographic linkages to seafloor and water column habitats, Front Microbiol, 4, -https://doi.org/10.3389/fmicb.2013.00124, 2013.

Djurhuus, A., Mikalsen, S. O., Giebel, H. A., and Rogers, A. D.: Cutting through the smoke: the diversity of microorganisms in deep-sea hydrothermal plumes, Royal Society Open Science, 4, https://doi.org/10.1098/rsos.160829, 2017.

Douville, E., Charlou, J. L., Oelkers, E. H., Bienvenu, P., Colon, C. F. J., Donval, J. P., Fouquet, Y., Prieur, D., and Appriou, P.: The rainbow vent fluids (36 degrees 14' N, MAR): the influence of ultramafic rocks and phase separation on trace metal content in Mid-Atlantic Ridge hydrothermal fluids, Chem Geol, 184, 37-48, https://doi.org/10.1016/S0009-2541(01)00351-5, 2002.

Dymond, J., and Roth, S.: Plume Dispersed Hydrothermal Particles - a Time-Series Record of Settling Flux from the Endeavor Ridge Using Moored Sensors, Geochim Cosmochim Ac, 52, 2525-2536, 1988.

Edmonds, H. N., and German, C. R.: Particle geochemistry in the Rainbow hydrothermal plume, Mid-Atlantic Ridge, Geochim Cosmochim Ac, 68, 759-772, https://doi.org/10.1016/S0016-7037(03)00498-810.1016/0016-7037(88)90310-9, 2004.

Emery, W. J., and Meincke, J.: Global Water Masses - Summary and Review, Oceanol Acta, 9, 383-391, 0399-1784/86/04, 1986.

Findlay, A. J., Gartman, A., Shaw, T. J., and Luther, G. W.: Trace metal concentration and partitioning in the first 1.5 m of hydrothermal vent plumes along the Mid-Atlantic Ridge: TAG, Snakepit, and Rainbow, Chem Geol, 412, 117-131, https://doi.org/10.1016/j.chemgeo.2015.07.021, 2015.

Fouquet, Y., Barriga, F., Charlou, J. L., Elderfield, H., German, C. R., Ondréas, H., Parson, L., Radford-Knoery, J., Relvas, J., Ribeiro, A., Schultz, A., Apprioual, R., Cambon, P., Costa, I., Donval, J. P., Douville, E., Landuré, J. Y., Normund, A., Pellé, H., Ponsevera, E., Riches, S., Santana, H., and Stephan, M.: Flores diving cruise with the Nautile near the Azores. First dives on the Rainbow field: hydrothermal seawater/mantle interaction, InterRidge News, 7, 24-28, 1998.

Frank, K. L., Rogers, D. R., Olins, H. C., Vidoudez, C., and Girguis, P. R.: Characterizing the distribution and rates of microbial sulfate reduction at Middle Valley hydrothermal vents, Isme J, 7, 1391-1401, https://doi.org/10.1038/ismej.2013.17, 2013.

789 German, C. R., Campbell, A. C., and Edmond, J. M.: Hydrothermal Scavenging at the Mid-Atlantic Ridge -
790 Modification of Trace-Element Dissolved Fluxes, Earth and Planetary Science Letters, 107, 101-114,
791 https://doi.org/10.1016/0012-821X(91)90047-L, 1991.

792 ~~German, C. R., and Sparks, R. S. J.: Particle Recycling in the Tag Hydrothermal Plume, Earth and Planetary Science~~
793 ~~Letters, 116, 129-134, https://doi.org/10.1016/0012-821X(93)90049-F, 1993.~~

794 German, C. R., Klinkhammer, G. P., and Rudnicki, M. D.: The Rainbow hydrothermal plume, 36 degrees 15'N,
795 MAR, Geophys Res Lett, 23, 2979-2982, https://doi.org/10.1029/96GL02883, 1996.

796 German, C. R., Richards, K. J., Rudnicki, M. D., Lam, M. M., Charlou, J. L., and Party, F. S.: Topographic control
797 of a dispersing hydrothermal plume, Earth and Planetary Science Letters, 156, 267-273,
798 https://doi.org/10.1016/S0012-821X(98)00020-X, 1998.

799 Gwyther, D., and Wright, M.: Environmental Impact Statement: Solwara 1, Coffey Natural Systems Pty Ltd, 47-
800 65, 2008.

801 Han, Y. C., Gonnella, G., Adam, N., Schippers, A., Burkhardt, L., Kurtz, S., Schwarz-Schampera, U., Franke, H.,
802 and Perner, M.: Hydrothermal chimneys host habitat-specific microbial communities: analogues for studying the
803 possible impact of mining seafloor massive sulfide deposits, Sci Rep-Uk, 8, https://doi.org/10.1038/s41598-018-
804 28613-5, 2018.

805 Huber, J. A., Butterfield, D. A. and Baross, J. A.: Bacterial diversity in a subseafloor habitat following a deep-sea
806 volcanic eruption. FEMS Microbiol Ecol, 43(3), pp.393-409, https://doi.org/10.1111/j.1574-6941.2003.tb01080.x,
807 2003.

808 Hoagland, P., Beaulieu, S., Tivey, M. A., Eggert, R. G., German, C., Glowka, L., and Lin, J.: Deep-sea mining of
809 seafloor massive sulfides, Mar Policy, 34, 728-732, https://doi.org/10.1016/j.marpol.2009.12.001, 2010.

810 Jannasch, H. W., and Mottl, M. J.: Geomicrobiology of Deep-Sea Hydrothermal Vents, Science, 229, 717-725,
811 https:/doi.org/10.1126/science.229.4715.717, 1985.

812 Jones, D. O. B., Amon, D. L., and Chapman, A. S. A.: Mining Deep-Ocean Mineral Deposits: What are the
813 Ecological Risks?, Elements, 14, 325-330, https://doi.org/10.2138/gselements.14.5.325, 2018.

~~Juniper, S. K., Bird, D. F., Summit, M., Vong, M. P., and Baker, E. T.: Bacterial and viral abundances in hydrothermal event plumes over northern Gorda Ridge, Deep Sea Res Pt Ii, 45, 2739-2749, https://doi.org/10.1016/S0967-0645(98)00091-5, 1998.~~

Khripounoff, A., Vangriesheim, A., Crassous, P., Segonzac, M., Colaco, A., Desbruyeres, D., and Barthelemy, R.: Particle flux in the Rainbow hydrothermal vent field (Mid-Atlantic Ridge): Dynamics, mineral and biological composition, J Mar Res, 59, 633-656, https://doi.org/10.1357/002224001762842217, 2001.

Levin, L. A., Mengerink, K., Gjerde, K. M., Rowden, A. A., Van Dover, C. L., Clark, M. R., Ramirez-Llodra, E., Currie, B., Smith, C. R., Sato, K. N., Gallo, N., Sweetman, A. K., Lily, H., Armstrong, C. W., and Brider, J.: Defining "Serious Harm" to the Marine Environment in the Context of Deep-Seabed Mining, Marine Policy, 245-59, http://dx.doi.org/10.1016/j.marpol.2016.09.032, 2016

Lopez-Garcia, P., Philippe, H., Gail, F., and Moreira, D.: Autochthonous eukaryotic diversity in hydrothermal sediment and experimental microcolonizers at the Mid-Atlantic Ridge, P Natl Acad Sci USA, 100, 697-702, https://doi.org/10.1073/pnas.0235779100, 2003.

Ludford, E. M., Palmer, M. R., German, C. R., and Klinkhammer, G. P.: The geochemistry of Atlantic hydrothermal particles, Geophys Res Lett, 23, 3503-3506, https://doi.org/10.1029/96GL02078, 1996.

Mandernack, K. W., and Tebo, B. M.: Manganese Scavenging and Oxidation at Hydrothermal Vents and in Vent Plumes, Geochim Cosmochim Ac, 57, 3907-3923, https://doi.org/10.1016/0016-7037(93)90343-U, 1993.

Marques, A. F. A., Barriga, F., Chavagnac, V. and Fouquet, Y. : Mineralogy, geochemistry, and Nd isotope composition of the Rainbow hydrothermal field, Mid-Atlantic Ridge, Mineralium Deposita, 41, 52-67, https://doi.org/10.007/s00126-005-0040-8, 2006.

McCollom, T. M.: Geochemical constraints on primary productivity in submarine hydrothermal vent plumes, Deep-Sea Res Pt I, 47, 85-101, https://doi.org/10.1016/S0967-0637(99)00048-5, 2000.

Michard, G., Albarède, F., Michard, A., Minister, J. F., Charlou. J. L., and Tan, N.: Chemistry of Solution from the 13 degrees N East Pacific Rise Hydrothermal Site. Earth and Planetary Science Letters 67, 297-307, https://doi.org/10.1016/0012-821X(84)90169-9, 1984

Mino, S., Nakagawa, S., Makita, H., Toki, T., Miyazaki, J., Sievert, S. M., ... & Watanabe, H.: Endemicity of the cosmopolitan mesophilic chemolithoautotroph Sulfurimonas at deep-sea hydrothermal vents. The ISME journal, 11(4), 909, https://doi.org/10.1038/ismej.2016.178, 2017.

Nakagawa, S., Takai, K., Inagaki, F., Hirayama, H., Nunoura, T., Horikoshi, K., and Sako, Y.: Distribution, phylogenetic diversity and physiological characteristics of epsilon-Proteobacteria in a deep-sea hydrothermal field, Environ Microbiol, 7, 1619-1632, https://doi.org/10.1111/j.1462-2920.2005.00856.x, 2005.

Olins, H. C., Rogers, D. R., Preston, C., Ussler, W., Pargett, D., Jensen, S., Roman, B., Birch, J. M., Scholin, C. A., Haroon, M. F., and Girguis, P. R.: Co-registered Geochemistry and Metatranscriptomics Reveal Unexpected Distributions of Microbial Activity within a Hydrothermal Vent Field, Front Microbiol, 8, https://doi.org/10.3389/fmicb.2017.01042, 2017.

Opatkiewicz, A. D., Butterfield, D. A., and Baross, J. A.: Individual hydrothermal vents at Axial Seamount harbor distinct subseafloor microbial communities, Fems Microbiol Ecol, 70, 413-424, https://doi.org/10.1111/j.1574-6941.2009.00747.x, 2009.

Orcutt, B. N., Sylvan, J. B., Knab, N. J., & Edwards, K. J.: Microbial ecology of the dark ocean above, at, and below the seafloor. Microbiol. Mol. Biol. Rev., 75(2), 361-422, https://doi.org/~~doi:~~10.1128/MMBR.00039-10, 2011.

Phillips, B. T.: Beyond the vent: New perspectives on hydrothermal plumes and pelagic biology, Deep-Sea Res Pt Ii, 137, 480-485, https://doi.org/10.1016/j.dsr2.2016.10.005, 2017.

Ramirez-Llodra, E., Tyler, P. A., Baker, M. C., Bergstad, O. A., Clark, M. R., Escobar, E., Levin, L. A., Menot, L., Rowden, A. A., Smith, C. R., and Van Dover, C. L.: Man and the Last Great Wilderness: Human Impact on the Deep Sea, Plos One, 6, https://doi.org/10.1371/journal.pone.0022588, 2011.

Reed, D. C., Breier, J. A., Jiang, H. S., Anantharaman, K., Klausmeier, C. A., Toner, B. M., Hancock, C., Speer, K., Thurnherr, A. M., and Dick, G. J.: Predicting the response of the deep-ocean microbiome to geochemical perturbations by hydrothermal vents, Isme J, 9, 1857-1869, https://doi.org/10.1038/ismej.2015.4, 2015.

Resing, J. A., P. N. Sedwick, C. R. German, W. J. Jenkins, J. W. Moffett, B. M. Sohst, and A. Tagliabue.: Basin-Scale Transport of Hydrothermal Dissolved Metals across the South Pacific Ocean. Nature 523, no. 7559, 200-3. http://dx.doi.org/10.1038/nature14577, 2015.

Severmann, S., Johnson, C. M., Beard, B. L., German, C. R., Edmonds, H. N., Chiba, H., and Green, D. R. H.: The effect of plume processes on the Fe isotope composition of hydrothermally derived Fe in the deep ocean as inferred from the Rainbow vent site, Mid-Atlantic Ridge, 36 degrees 14' N, Earth and Planetary Science Letters, 225, 63-76, https://doi.org/10.1016/j.epsl.2004.06.001, 2004.

Sheik, C. S., Anantharaman, K., Breier, J. A., Sylvan, J. B., Edwards, K. J., and Dick, G. J.: Spatially resolved sampling reveals dynamic microbial communities in rising hydrothermal plumes across a back-arc basin, Isme J, 9, 1434-1445, https://doi.org/10.1038/ismej.2014.228, 2015.

Sunamura, M., Higashi, Y., Miyako, C., Ishibashi, J., and Maruyama, A.: Two bactetia phylotypes are predominant in the Suiyo Seamount hydrothermal plume, Appl Environ Microb, 70, 1190-1198, https://doi.org/10.1128/AEM.70.2.1190-1198.2004, 2004.

Sylvan, J. B., Pyenson, B. C., Rouxel, O., German, C. R., & Edwards, K. J.: Time-series analysis of two hydrothermal plumes at 9° 50′ N East Pacific Rise reveals distinct, heterogeneous bacterial populations. Geobiology, 10(2), 178-192, https://doi.org/10.1111/j.1472-4669.2011.00315.x, 2012.

Thurnherr, A. M., and Richards, K. J.: Hydrography and high-temperature heat flux of the Rainbow hydrothermal site (36 degrees 14 ' N, Mid-Atlantic Ridge), J Geophys Res-Oceans, 106, 9411-9426, https://doi.org/10.1029/2000JC900164, 2001.

Thurnherr, A. M., Richards, K. J., German, C. R., Lane-Serff, G. F., and Speer, K. G.: Flow and mixing in the rift valley of the Mid-Atlantic Ridge, J Phys Oceanogr, 32, 1763-1778, https://doi.org/10.1175/1520-0485(2002)032<1763:FAMITR>2.0.CO;2, 2002.

Trocine, R. P. and Trefry, J. H.: Distribution and chemistry of suspended particles from an active hydrothermal vent site on the Mid-Atlantic Ridge at 26 °N, Earth and Planetary Science Letters 88, 1-15, https://doi.org/10.1016/0012-821X(88)90041-6, 1988.

van Bleijswijk, J. D. L., Whalen, C., Duineveld, G. C. A., Lavaleye, M. S. S., Witte, H. J., and Mienis, F.: Microbial assemblages on a cold-water coral mound at the SE Rockall Bank (NE Atlantic): interactions with hydrography and topography, Biogeosciences, 12, 4483-4496, https://doi.org/10.5194/bg-12-4483-2015, 2015.

van Haren, H., Duineveld, G., and de Stigter, H.: Prefrontal bore mixing, Geophys Res Lett, 44, 9408-9415, https://doi.org/10.1002/2017GL074384, 2017.

Vare, L. L., Baker, M. C., Howe, J. A., Levin, L. A., Neira, C., Ramirez-Llodra, E. Z., Reichelt-Brushett, A., Rowden, A. A., Shimmield, T. M., Simpson, S. L., and Soto, E. H.: Scientific Considerations for the Assessment and Management of Mine Tailings Disposal in the Deep Sea, Frontiers in Marine Science, 5, https://doi.org/10.3389/fmars.2018.00017, 2018.

~~Von Damm, K. L.: Evolution of the hydrothermal system at East Pacific Rise 9 50 N: geochemical evidence for changes in the upper oceanic crust, Mid-Ocean Ridges: Hydrothermal Interactions Between the Lithosphere and Oceans, 148, 285-304, https://doi.org/10.1029/148GM12, 2004.~~

Weaver, P. P., Billett, D. S., and Van Dover, C. L.: Environmental risks of deep-sea mining, in: Handbook on Marine Environment Protection, Springer, 215-245, https://doi.org/10.1007/978-3-319-60156-4_11, 2018.

Wetzel, L. R., and Shock, E. L.: Distinguishing ultramafic- from basalt-hosted submarine hydrothermal systems by comparing calculated vent fluid compositions, J Geophys Res-Sol Ea, 105, 8319-8340, https://doi.org/10.1029/1999JB900382, 2000.

~~Winn, C. D., Karl, D. M., and Massoth, G. J.: Microorganisms in Deep Sea Hydrothermal Plumes, Nature, 320, 744-746, https://doi.org/10.1038/320744a0, 1986.~~

Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W. and Glöckner, F.O.: The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. Nucleic Acids Res, 42(D1), pp.D643-D648, https://doi.org/10.1093/nar/gkt1209, 2014.

Zinger, L., Amaral-Zettler, L. A., Fuhrman, J. A., Horner-Devine, M. C., Huse, S. M., Welch, D. B. M., Martiny, J. B. H., Sogin, M., Boetius, A., and Ramette, A.: Global Patterns of Bacterial Beta-Diversity in Seafloor and Seawater Ecosystems, Plos One, 6, https://doi.org/10.1371/journal.pone.0024570, 2011.

Figures and tables

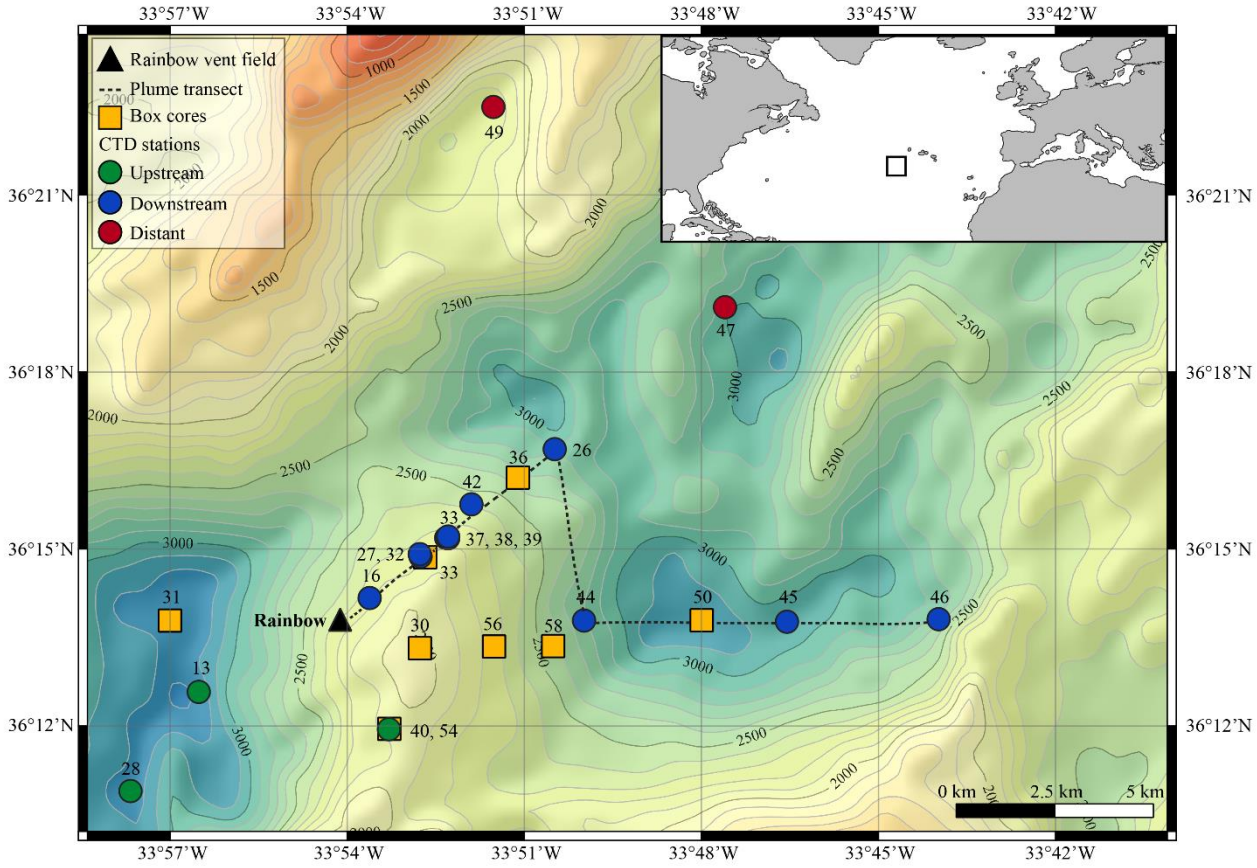


Figure 1: Geographical location (inset) and bathymetric map of the Rainbow study site on the Mid Atlantic Ridge ~~bathymetry (from~~ EMOD ~~data base)~~ with ~~Geographical location (inset), showing~~ sampling ~~methods and~~ locations depicted.

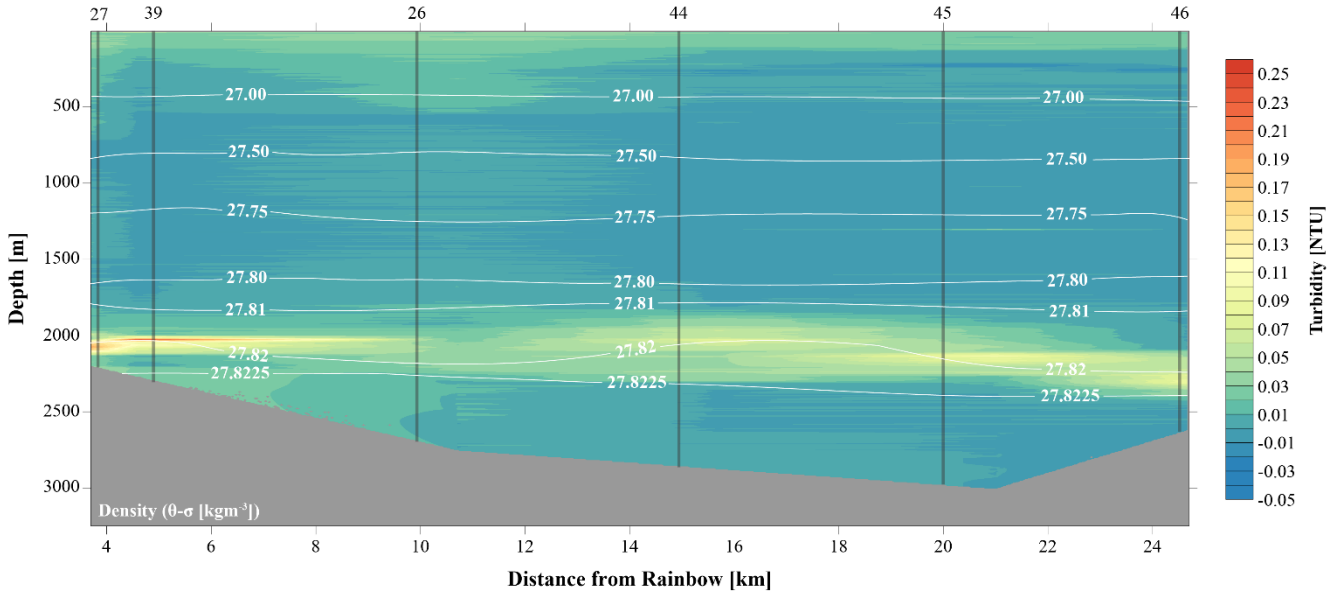


Figure 2: Transect along main plume path (indicated in Fig. 1 as plume transect), showing turbidity in the water column. The plume is indicated by highest turbidity values and disperses away from the Rainbow vent field.

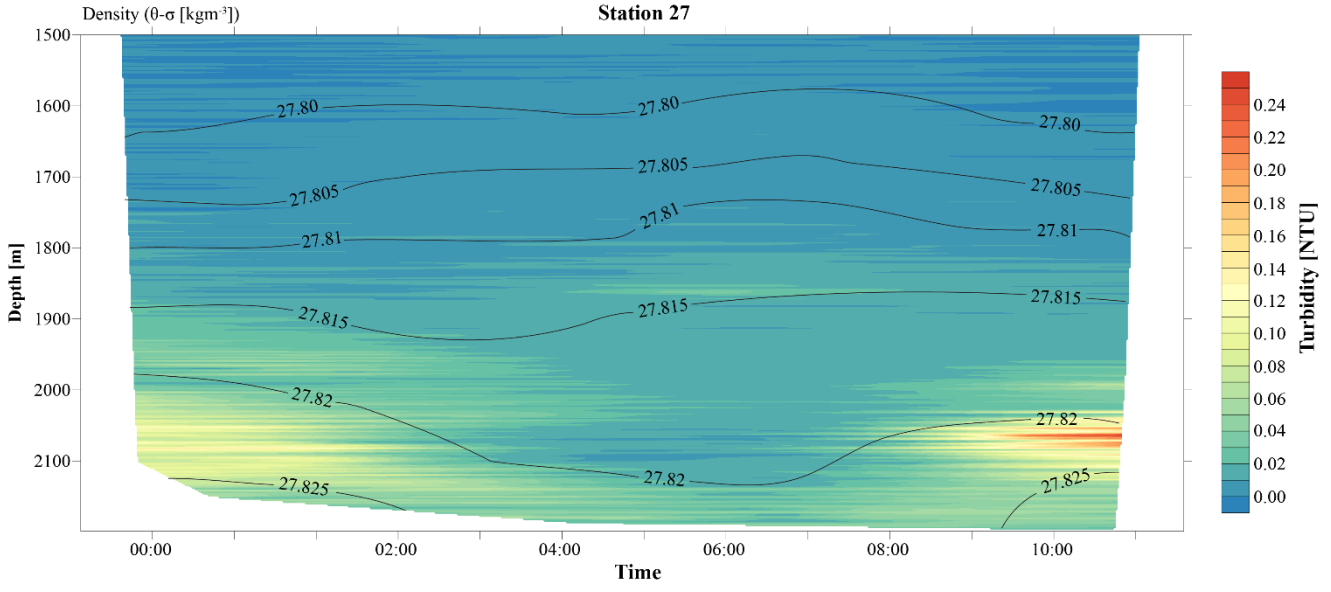


Figure 3: 12 hour CTD YOYO casts at station 27 showing the temporal evolution of the hydrothermal plume over a tidal cycle.

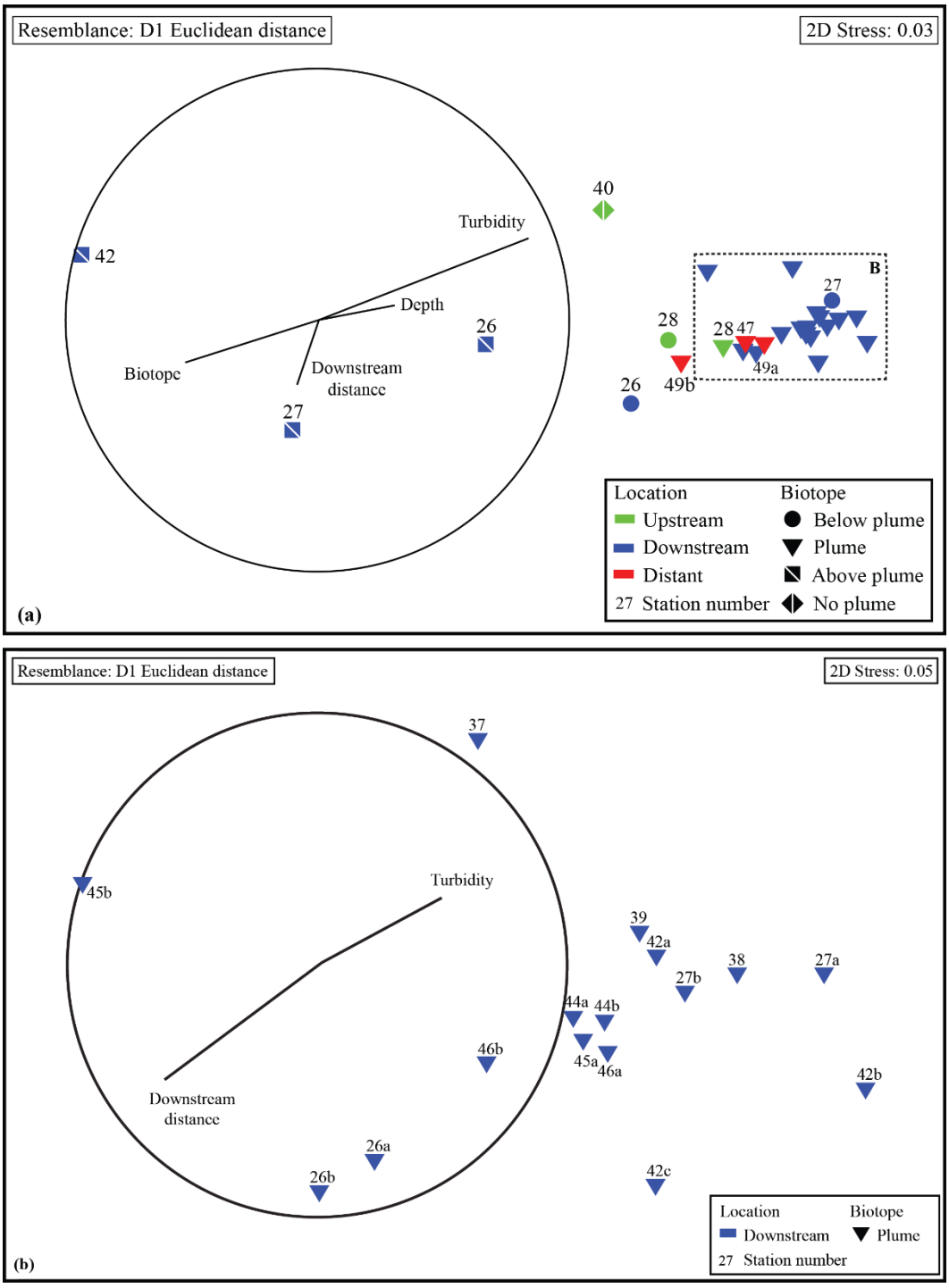


Figure 4: (a) NMDS ordination showing all water samples based on their resemblance in chemical composition. (b) NMDS ordination showing all plume samples from the downstream stations based on their resemblance in chemical composition.

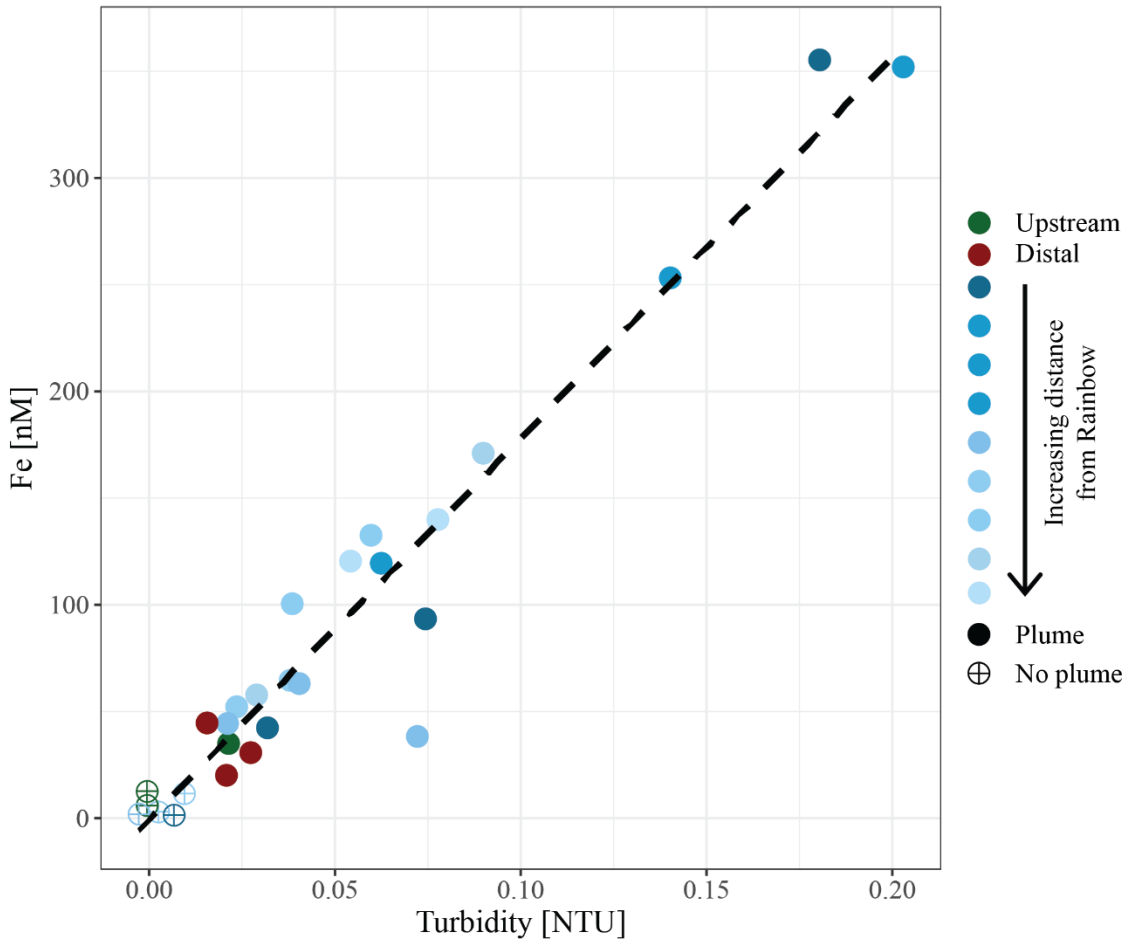
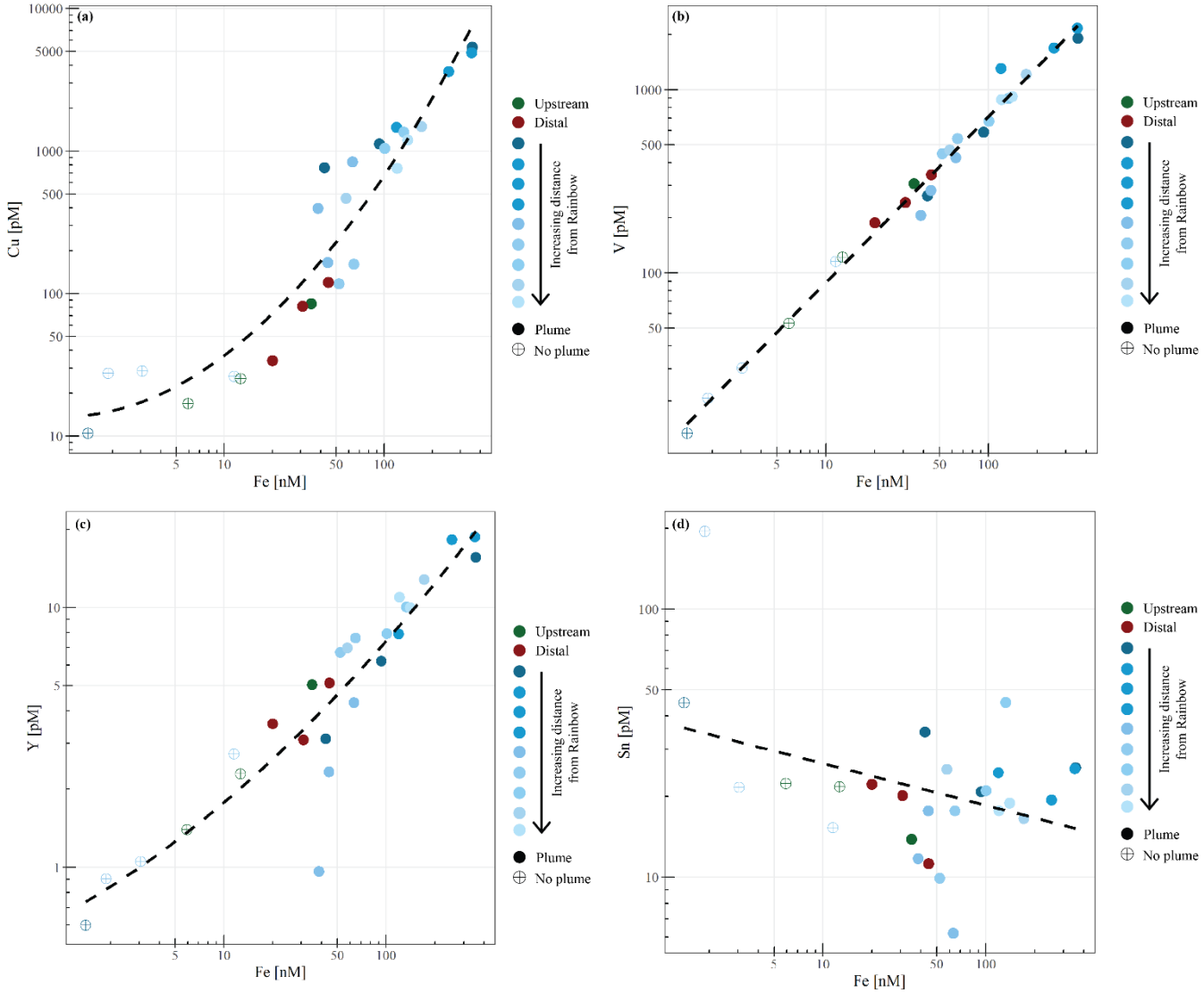


Figure 5: Relationship between in-situ measured turbidity and molar concentration of particulate iron.



936

937 *Figure 6: Relationships between molar concentrations of particulate copper (a), vanadium (b), yttrium (c) and*
938 *tin (d) to iron.*

939

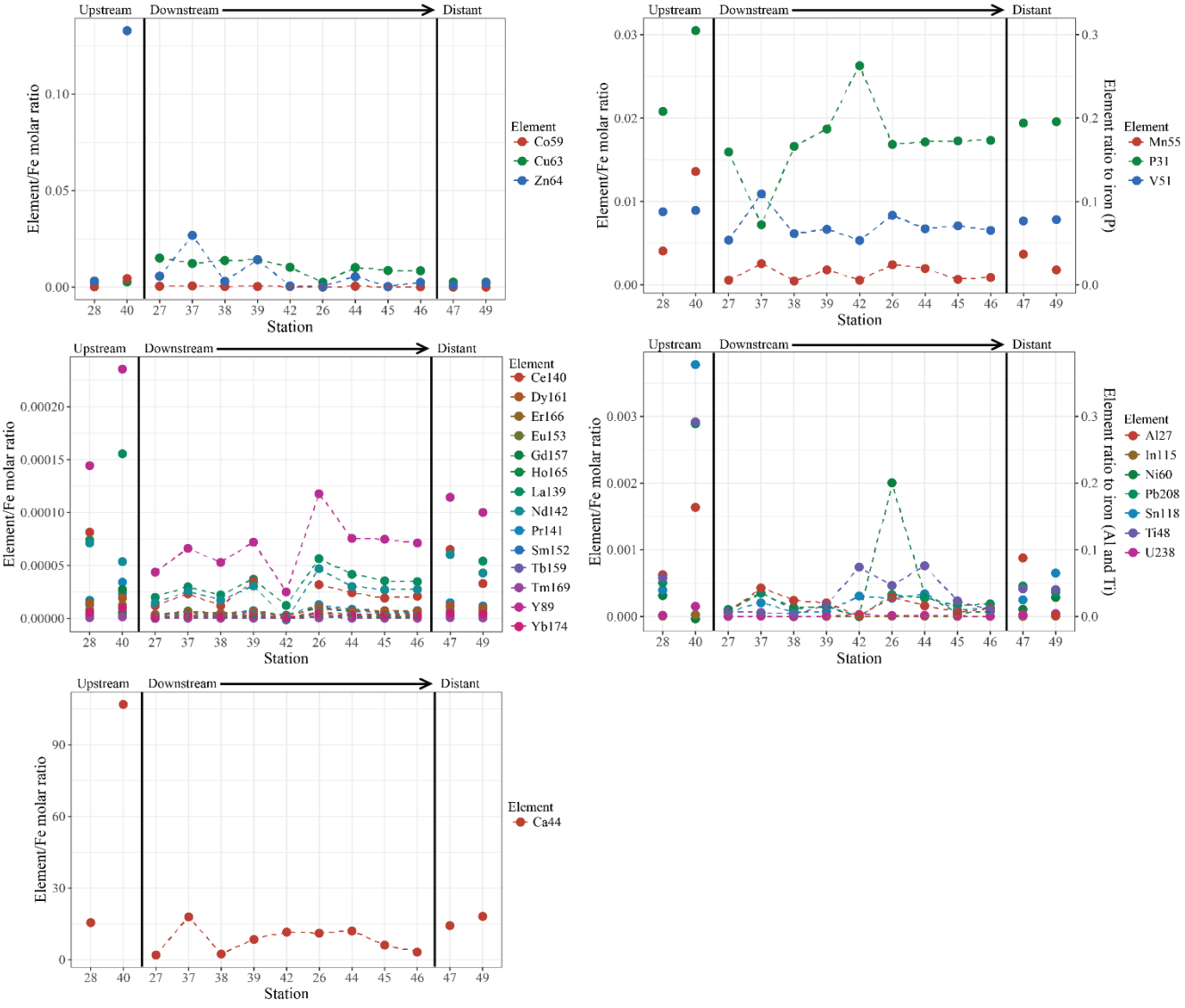


Figure 7: Element to iron molar ratios. Plume samples of upstream, downstream and distant stations. Downstream stations follow the main path of the plume. Fig. 7a) shows the element/Fe molar ratios of the chalcophiles (Co, Cu and Zn), b) shows the ratios of Mn and the oxyanions (P and V), c) displays the ratios of REE, d) the ratios of Al, In, Ni, Pb, Sn, Ti and U and e) shows the Ca/Fe molar ratio.

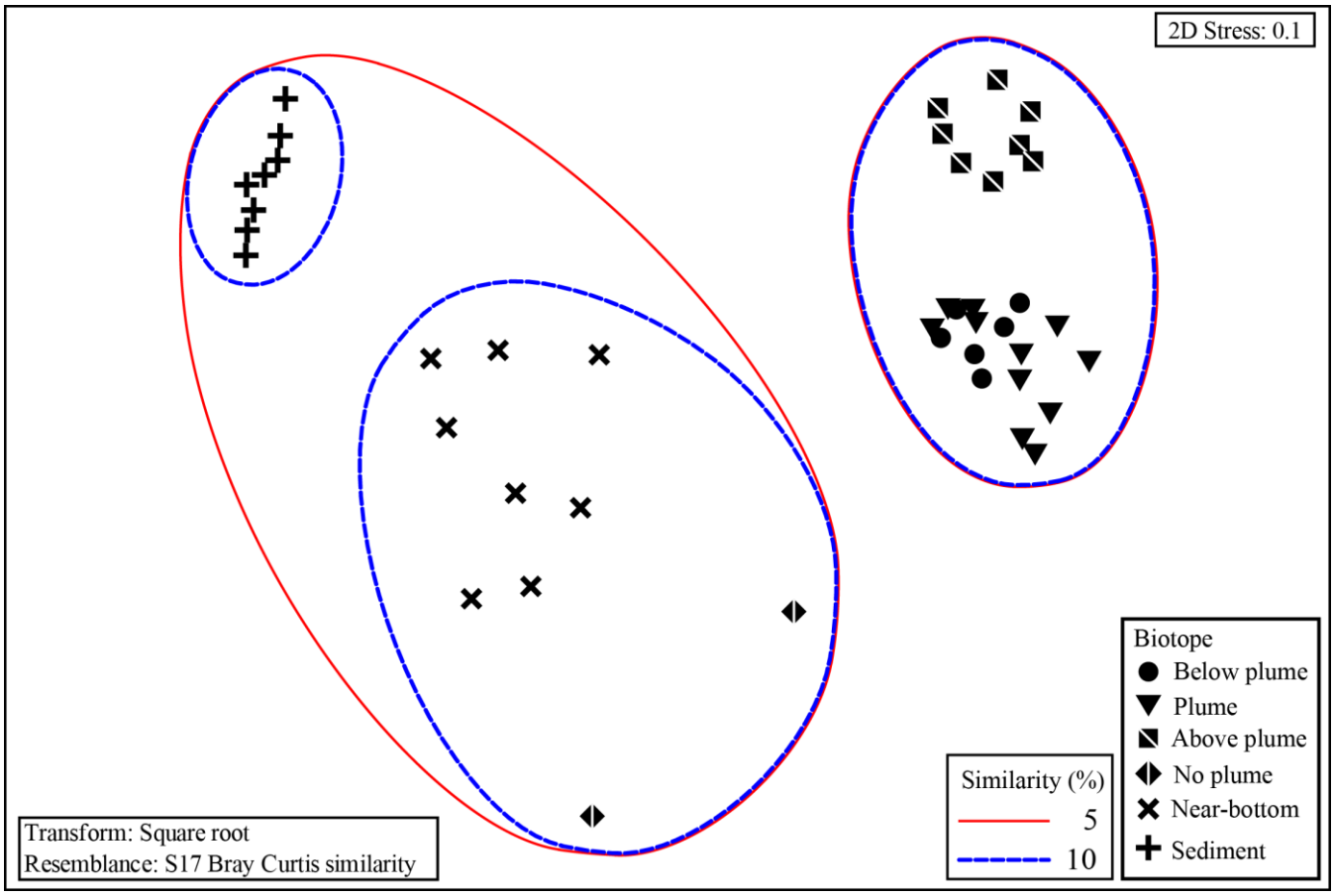


Figure 8: Non-metric multidimensional scaling plot of the microbial community composition of all samples based on Operational Taxonomic units. Similarity groupings are based on group average clustering. "No plume" is representative of samples collected from station 13, where there was no indication of a plume.

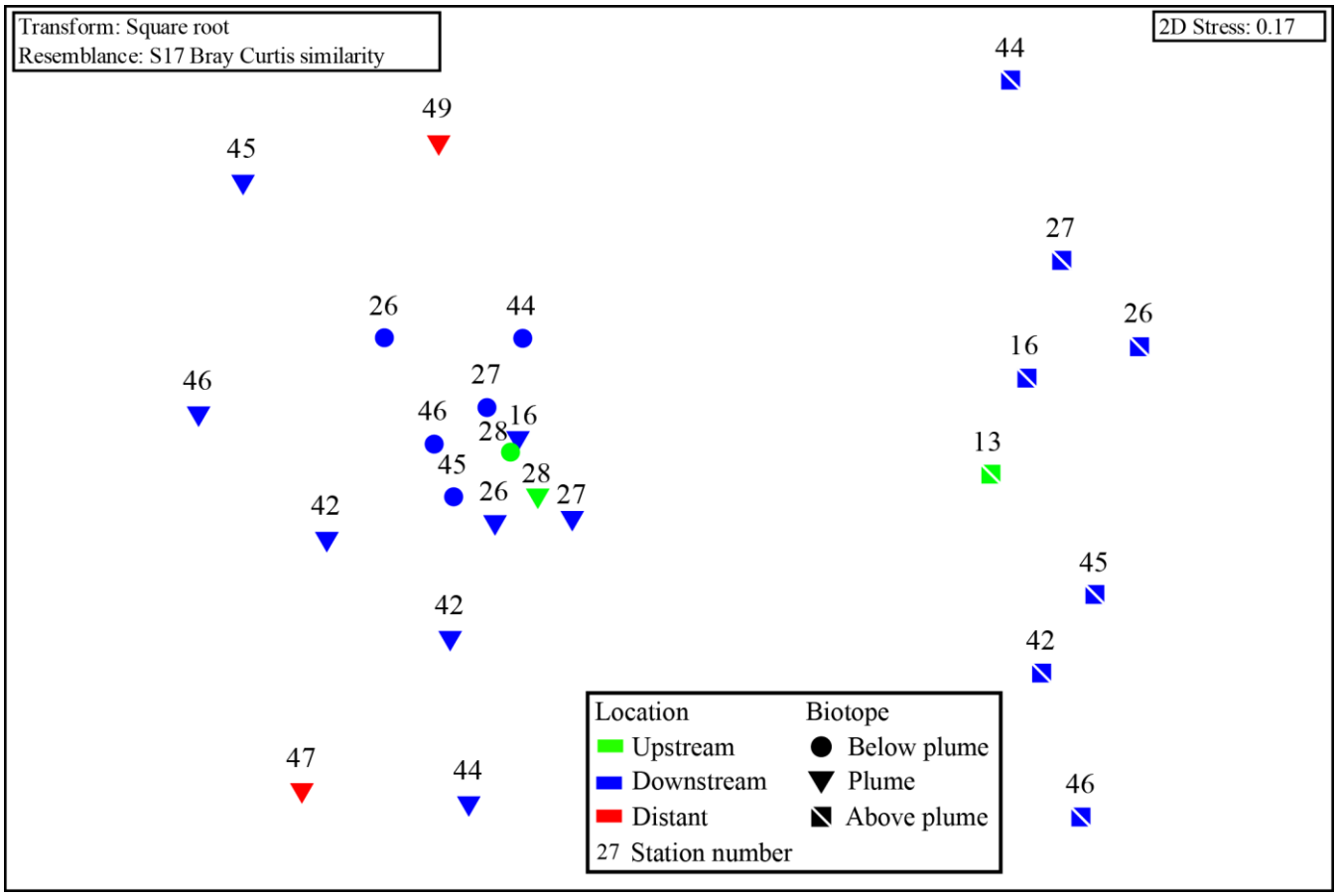


Figure 9: Non-metric multidimensional scaling plot of the microbial community composition of all water column samples based on Operational Taxonomic units. Plume and below plume depths from Station 13 were excluded.

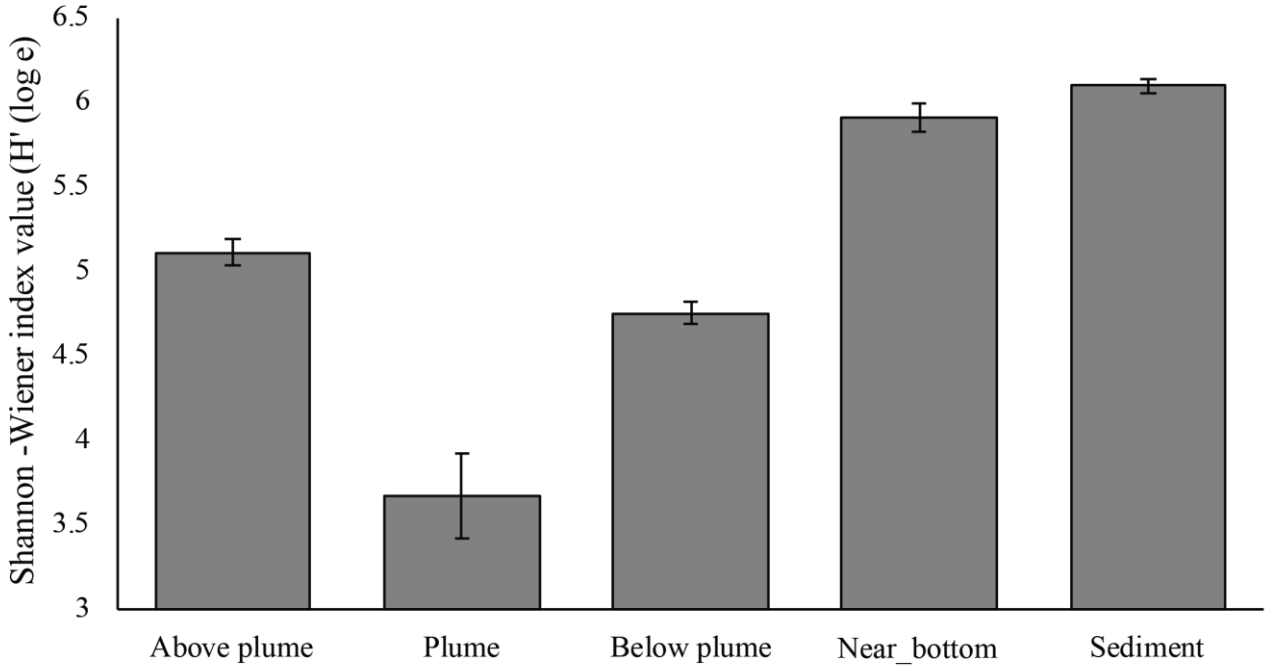


Figure 10: Mean Shannon-Wiener diversity index for microorganisms in each biotope. Error bars represent ±SE

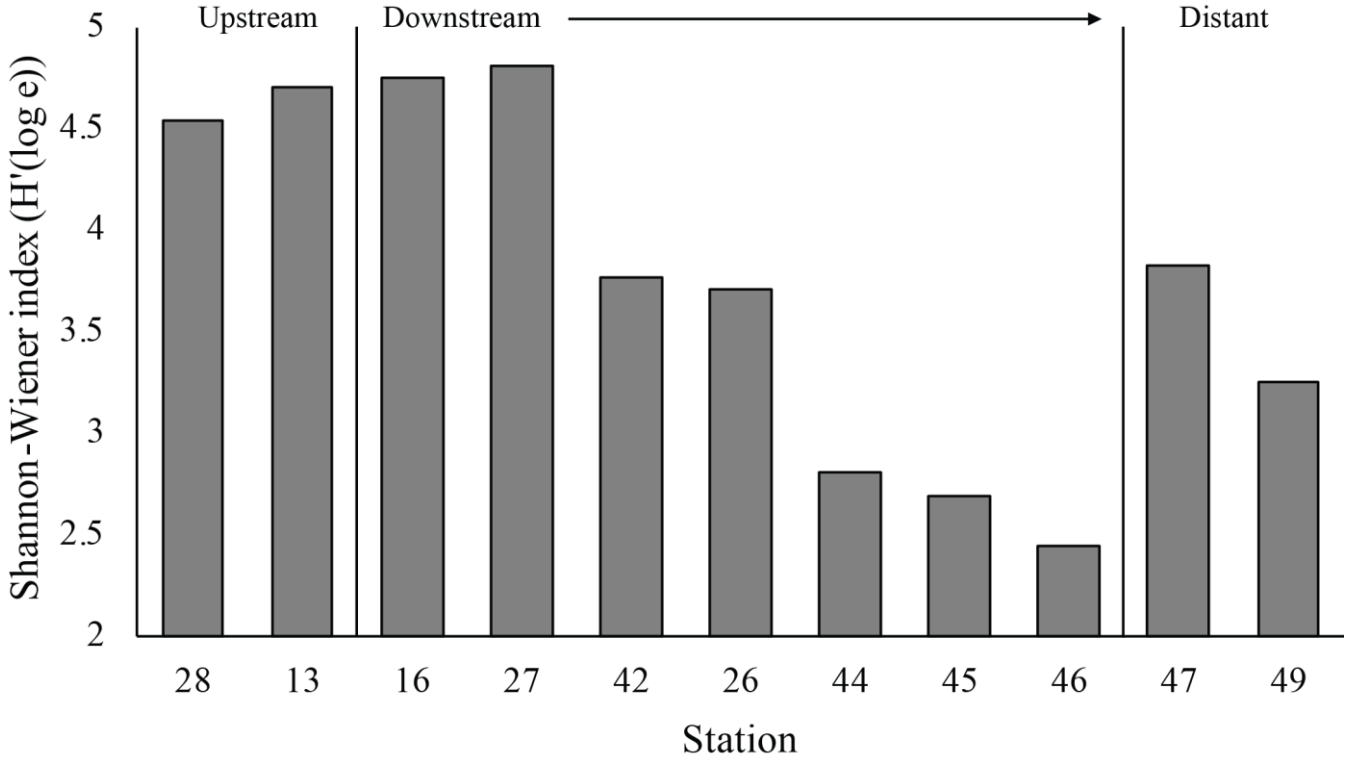
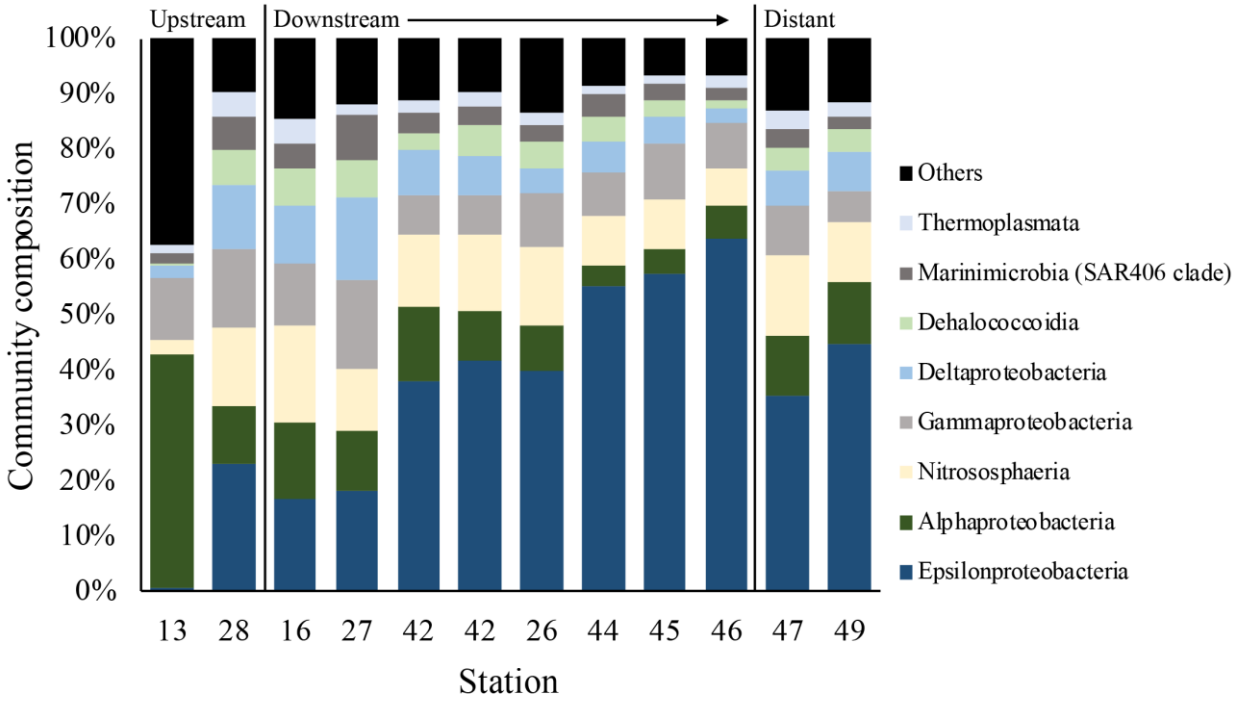


Figure 11: Shannon-Wiener index values for microorganisms in each plume sample taken.



961

962 *Figure 12: Microbial community composition in the plume samples as a percentage of the dominant class groups*
963 *in accordance with the SIMPER results.*

964

Table 1: Meta-data of samples taken.

Station	Latitude	Longitude	Biotope	Sample type	Depth (m)	Micro-biology	SPM	(Trace) metals
30	36°13’19”N	33°52’46”W	Sediment and near-bottom water	Box core	1970	x		
31	36°13’47”N	33°57’00”W	Sediment and near-bottom water	Box core	3190	x		
33	36°14’51”N	33°52’41”W	Sediment and near-bottom water	Box core	2223	x		
36	36°16’13”N	33°51’06”W	Sediment and near-bottom water	Box core	2857	x		
50	36°13’47”N	33°47’60”W	Sediment and near-bottom water	Box core	3157	x		
54	36°11’57”N	33°53’46”W	Sediment and near-bottom water	Box core	2129	x		
56	36°13’21”N	33°51’31”W	Sediment and near-bottom water	Box core	2198	x		
58	36°13’21”N	33°50’31”W	Sediment and near-bottom water	Box core	2514	x		
13	36°12’35”N	33°56’31”W	Above plume	CTD	125	x		
13	36°12’35”N	33°56’31”W	Below plume	CTD	3220	x		
13	36°12’35”N	33°56’31”W	Plume	CTD	2000	x		
16	36°14’10”N	33°53’37”W	Plume	CTD	1944	x		
16	36°14’10”N	33°53’37”W	Above plume	CTD	998	x		
26	36°16’41”N	33°50’29”W	Below plume	CTD	2756	x	x	x
26~~a~~	36°16’41”N	33°50’29”W	Plume	CTD	2150	x	x	x
26~~b~~	36°16’41”N	33°50’29”W	Plume	CTD	2000		x	x
26	36°16’41”N	33°50’29”W	Above plume	CTD	999	x	x	x
27	36°16’52”N	33°52’45”W	Below plume	CTD	2191	x		x
27~~a~~	36°16’52”N	33°52’45”W	Plume	CTD	2077	x		x
27~~b~~	36°16’52”N	33°52’45”W	Plume	CTD	1996			x
27	36°16’52”N	33°52’45”W	Above plume	CTD	994	x		x
28	36°10’54”N	33°57’40”W	Below plume	CTD	3170	x	x	x
28	36°10’54”N	33°57’40”W	Plume	CTD	1975	x	x	x
32~~a~~	36°14’55”N	33°52’46”W	Plume	CTD	2192		x	
32~~b~~	36°14’55”N	33°52’46”W	Plume	CTD	2088		x	
37	36°15’11”N	33°52’19”W	Plume	CTD	2190			x
38	36°15’11”N	33°52’17”W	Plume	CTD	2040			x
39	36°15’13”N	33°52’17”W	Plume	CTD	2019			x
40	36°11’57”N	33°53’18”W	No plume	CTD	2120			x
42~~a~~	36°15’45”N	33°51’54”W	Plume	CTD	2291	x	x	x
42~~b~~	36°15’45”N	33°51’54”W	Plume	CTD	2209	x	x	x
42~~c~~	36°15’45”N	33°51’54”W	Plume	CTD	2037		x	x
42	36°15’45”N	33°51’54”W	Above plume	CTD	999	x	x	x
44	36°13’47”N	33°49’59”W	Below plume	CTD	2623	x		
44~~a~~	36°13’47”N	33°49’59”W	Plume	CTD	2202		x	x
44~~b~~	36°13’47”N	33°49’59”W	Plume	CTD	2002	x	x	x
44	36°13’47”N	33°49’59”W	Above plume	CTD	995	x		
45	36°13’46”N	33°46’33”W	Below plume	CTD	3004	x		
45~~a~~	36°13’46”N	33°46’33”W	Plume	CTD	2166		x	x
45~~b~~	36°13’46”N	33°46’33”W	Plume	CTD	2002	x	x	x
45	36°13’46”N	33°46’33”W	Above plume	CTD	996	x		
46	36°13’49”N	33°43’59”W	Below plume	CTD	2622	x		
46~~a~~	36°13’49”N	33°43’59”W	Plume	CTD	2280	x	x	x
46~~b~~	36°13’49”N	33°43’59”W	Plume	CTD	2145		x	x
46	36°13’49”N	33°43’59”W	Above plume	CTD	1000	x		
47	36°19’06”N	33°47’36”W	Below plume	CTD	2850			
47	36°19’06”N	33°47’36”W	Plume	CTD	2200	x		x
49~~a~~	36°22’19”N	33°51’31”W	Plume	CTD	2260	x	x	x
49~~b~~	36°22’19”N	33°51’31”W	Plume	CTD	1902		x	x

967 *Table 2: Primers used for sequencing.*

Forward		Reverse			
Primer name	Primer sequence 5'-3'	Primer name	Primer sequence 5'-3'	Ratio in mix	Reference
Arch-0519-a-S-1 (universal)	CAGCMGCCGCGGTAA	Bact-0785-b-A-18 (universal)	TACNVGGGTATCTAATCC	3/9 + 3/9	Klindworth et al. 2012
Bact-0519F (targets WS6, TM7, OP11	CAGCAGCATCGGTVA			1/9	This paper
Nano-0519F (targets Nanoarchaea)	CAGTCGCCRCGGGAA	Nano-0785R (targets Nanoarchaea)	TACNVGGGTMTCTAATYY	1/9+1/9	This paper

968

969

970 *Table 3: SIMPER similarity results of each biotope at class level. ** undefined class.*

Biotope	Average similarity (%)	Class	Average proportion (%)	Average similarity	Sim/SD	Contribution (%)	Cumulative %
Above plume	82.34	Nitrososphaeria	27.10	22.79	4.61	27.67	27.67
		Alphaproteobacteria	18.34	15.22	4.15	18.49	46.16
		Gammaproteobacteria	13.44	11.58	5.52	14.07	60.23
		Deltaproteobacteria	10.67	8.46	3.38	10.27	70.50
		Marinimicrobia (SAR406 clade)	8.22	6.96	6.07	8.46	78.96
		Dehalococcoidia	6.38	5.69	9.19	6.91	85.87
		Thermoplasmata	2.63	2.26	5.68	2.74	88.61
		Acidimicrobiia	2.13	1.89	8.62	2.30	90.91
Plume	76.74	Epsilonproteobacteria	39.59	30.29	2.53	39.47	39.47
		Nitrososphaeria	12.16	10.32	4.05	13.45	52.92
		Gammaproteobacteria	9.69	7.92	4.71	10.32	63.23
		Alphaproteobacteria	9.23	7.22	2.44	9.40	72.64
		Deltaproteobacteria	7.60	5.56	2.75	7.25	79.88
		Dehalococcoidia	4.57	3.55	2.58	4.63	84.51
		Marinimicrobia (SAR406 clade)	4.02	3.07	3.83	4.00	88.51
		Thermoplasmata	2.56	1.94	3.39	2.53	91.04
Below plume	77.94	Nitrososphaeria	22.35	16.60	3.29	21.30	21.30
		Alphaproteobacteria	13.26	11.43	5.18	14.67	35.97
		Deltaproteobacteria	10.88	9.25	8.31	11.87	47.84
		Gammaproteobacteria	10.60	8.89	7.78	11.40	59.24
		Epsilonproteobacteria	9.65	7.18	2.50	9.22	68.46
		Dehalococcoidia	7.84	6.97	7.89	8.95	77.40
		Marinimicrobia (SAR406	6.32	4.49	2.31	5.76	83.16
		Thermoplasmata	4.69	3.04	2.20	3.90	87.07
		Phycisphaerae	1.97	1.75	7.60	2.24	89.31
		Planctomycetacia	2.03	1.50	2.96	1.93	91.23
Near-bottom water	75.71	Gammaproteobacteria	20.79	16.77	3.18	22.15	22.15
		Nitrososphaeria	16.90	13.54	3.79	17.89	40.04
		Alphaproteobacteria	15.55	13.25	5.47	17.50	57.54
		Deltaproteobacteria	6.68	5.89	5.99	7.78	65.32
		Oxyphotobacteria	5.93	4.04	2.18	5.34	70.66
		Dehalococcoidia	4.08	2.99	2.50	3.95	74.61
		Phycisphaerae	3.72	2.57	2.03	3.40	78.01
		Thermoplasmata	2.47	1.70	2.25	2.24	80.25
		Acidimicrobiia	2.06	1.61	2.72	2.13	82.38
		Bacteroidia	2.15	1.57	1.85	2.07	84.45
		Marinimicrobia (SAR406 clade)	1.75	1.24	2.17	1.64	86.09
		OM190	1.64	1.14	2.02	1.51	87.60
		Planctomycetacia	1.40	1.09	2.76	1.44	89.04
		Epsilonproteobacteria	1.71	0.85	1.08	1.12	90.16
Sediment	82.51	Gammaproteobacteria	29.67	27.17	8.51	32.93	32.93
		Alphaproteobacteria	13.98	12.44	4.88	15.07	48.01
		Deltaproteobacteria	11.98	10.98	10.24	13.30	61.31
		Nitrososphaeria	7.73	5.69	3.74	6.90	68.21
		Phycisphaerae	5.46	5.01	7.85	6.07	74.28
		Dehalococcoidia	3.35	2.48	2.58	3.01	77.29
		BD2-11 terrestrial group	2.36	1.91	2.90	2.31	79.60
		Subgroup 22 (Acidobacteria)	2.10	1.74	3.22	2.11	81.71
		OM190	2.09	1.50	5.50	1.81	83.53
		Nitrospira	1.79	1.49	3.68	1.80	85.33
		Bacteroidia	1.91	1.48	3.66	1.79	87.12
		Acidimicrobiia	1.58	1.24	2.84	1.50	88.62
		Thermoanaerobaculia	1.41	1.07	3.25	1.30	89.92
		Gemmatimonadetes**	1.57	1.06	1.56	1.28	91.21

971

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 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 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 <u>can be characterised by means of geochemistry and microbiology</u> 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 down 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 ^{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 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."

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.

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 Fer 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: Chemiolithoautotrophic?

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^{-1} (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

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.