

ORIGINAL ARTICLE

Predicting the response of the deep-ocean microbiome to geochemical perturbations by hydrothermal vents

Daniel C Reed¹, John A Breier², Houshuo Jiang², Karthik Anantharaman¹, Christopher A Klausmeier³, Brandy M Toner⁴, Cathrine Hancock⁵, Kevin Speer^{5,6}, Andreas M Thurnherr⁷ and Gregory J Dick¹

¹Department of Earth and Environmental Sciences, University of Michigan, Ann Arbor, MI, USA;

²Department of Ocean Physics and Engineering, Woods Hole Oceanographic Institution, Woods Hole, MA, USA; ³Kellogg Biological Station, Department of Plant Biology, Michigan State University, Hickory Corners, MI, USA; ⁴Department of Soil, Water, and Climate, University of Minnesota-Twin Cities, St. Paul, MN, USA;

⁵Geophysical Fluid Dynamics Institute, Florida State University, Tallahassee, FL, USA; ⁶Department of Earth, Ocean and Atmospheric Science, Florida State University, Tallahassee, FL, USA and ⁷Division of Ocean and Climate Physics, Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY, USA

Submarine hydrothermal vents perturb the deep-ocean microbiome by injecting reduced chemical species into the water column that act as an energy source for chemosynthetic organisms. These systems thus provide excellent natural laboratories for studying the response of microbial communities to shifts in marine geochemistry. The present study explores the processes that regulate coupled microbial-geochemical dynamics in hydrothermal plumes by means of a novel mathematical model, which combines thermodynamics, growth and reaction kinetics, and transport processes derived from a fluid dynamics model. Simulations of a plume located in the ABE vent field of the Lau basin were able to reproduce metagenomic observations well and demonstrated that the magnitude of primary production and rate of autotrophic growth are largely regulated by the energetics of metabolisms and the availability of electron donors, as opposed to kinetic parameters. Ambient seawater was the dominant source of microbes to the plume and sulphur oxidisers constituted almost 90% of the modelled community in the neutrally-buoyant plume. Data from drifters deployed in the region allowed the different time scales of metabolisms to be cast in a spatial context, which demonstrated spatial succession in the microbial community. While growth was shown to occur over distances of tens of kilometers, microbes persisted over hundreds of kilometers. Given that high-temperature hydrothermal systems are found less than 100 km apart on average, plumes may act as important vectors between different vent fields and other environments that are hospitable to similar organisms, such as oil spills and oxygen minimum zones.

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Introduction

Microbial life is abundant throughout the oceans and has a key role in modulating global geochemical cycles (Falkowski *et al.*, 2008). The advent of advanced molecular tools has provided an invaluable means of studying spatial and temporal variations in the phylogenetic structure and geochemical function of these marine microbial communities (Gilbert and Dupont, 2011). Such insights are critical for predicting the future of the oceans

given that anthropogenic perturbations, like climate change and coastal eutrophication, are causing marine ecosystems to deviate from their natural states with uncertain consequences (Nogales *et al.*, 2013). While mathematical models are often used to make such predictions (for example, IPCC, 2013)—as well as to elucidate underlying mechanisms and to quantify processes—a current challenge is incorporating data from cutting-edge molecular tools into biogeochemical models.

The present communication focuses on predicting the response of microbial communities to geochemical perturbations in the deep ocean, which remains an enigmatic environment despite constituting the largest ecosystem on Earth (Aristegui *et al.*, 2009). In contrast to sunlit surface waters, primary production in the aphotic zone is typically energy limited. Deep-sea autotrophs generate energy by mediating

Correspondence: DC Reed, Department of Earth and Environmental Sciences, University of Michigan, 1100 North University Avenue, 2534 CC Little Building, Ann Arbor, MI 48109-1005, USA.

E-mail: reeddc@umich.edu

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redox reactions and, given that the oceans are largely oxygenated, growth is chiefly regulated by the availability of electron donors (Jannasch and Wirsen, 1979). Hydrothermal systems, located along mid-ocean ridges, are a major source of these electron donors to the deep ocean. In these regions, hot fluids rich in reduced chemical species rise from beneath the seafloor and are injected into the cold, oxic water column (Tivey, 2004), stimulating microbial growth as a result (Winn *et al.*, 1986). These natural geochemical perturbations thus provide an excellent opportunity for studying marine microbial dynamics. Chemosynthetic organisms support extensive biological communities at hydrothermal vents (Grassle, 1986). Nevertheless, their impact is not confined to the vent locale as hydrothermal plumes distribute material throughout the oceans as they rise, spread laterally, and become entrained in deep circulation (Tagliabue *et al.*, 2010). Plumes may thus act as vectors, linking microbial communities in various marine environments (Dick *et al.*, 2013). While recent metagenomic and metatranscriptomic studies have provided much-needed insight into those microbes present in plumes as well as their metabolisms (Baker *et al.*, 2012; Lesniewski *et al.*, 2012; Anantharaman *et al.*, 2013; Baker *et al.*, 2013; Marshall and Morris, 2013; Li *et al.*, 2014; Sheik *et al.*, in press), fundamental questions remain regarding the ecology and dynamics of these communities. Both near-vent and pelagic communities have been invoked as the origin of plume-dwelling microbes (Winn *et al.*, 1986; Lesniewski *et al.*, 2012), but how these potential sources interact with plume physics, vent chemistry and microbial growth to shape plume communities remains unclear.

Given that energy drives deep-sea autotrophy, thermodynamic mixing models have been used extensively over the past 20 years to estimate primary production in chimney walls, plumes and low-temperature diffuse flow regions of submarine hydrothermal systems (for example, Shock *et al.*, 1995; McCollom and Shock, 1997; McCollom, 2000, 2007; Amend *et al.*, 2011; Anantharaman *et al.*, 2013; Boettger *et al.*, 2013; Anantharaman *et al.*, 2014; Nakamura and Takai, 2014). These models calculate the amount of energy that is potentially available through a wide range of pathways, thus predicting the prevalence of these metabolisms. More recently, a reactive-transport model (RTM) of an ideal hydrothermal chimney was developed that not only considers thermodynamics, like previous models, but also explicitly incorporates reaction kinetics, allowing processes that occur within chimney walls to be spatially resolved (LaRowe *et al.*, 2014). These studies have provided great insight into the microbial metabolisms that are potentially active within hydrothermal systems, but are largely theoretical and do not make detailed, site-specific comparisons with microbial data. A recent study has sought to address this issue by

developing a metastable equilibrium model that was fit directly to phylum-level microbial data from a hydrothermal spring in Yellowstone National Park (Dick and Shock, 2013). However, while this approach is able to reproduce observations well, it is unable to forge links between the microbial community and geochemical dynamics since metabolic function cannot be assigned based on phyla and the model does not explicitly include biogeochemical processes.

Recently, a modelling framework was advanced that couples marine microbiology and geochemistry by directly linking microbial growth, reaction kinetics and thermodynamics (Reed *et al.*, 2014). The microbial community is divided according to metabolism and these sub-populations are tracked using associated functional genes. Previously, this method has been successfully applied to study the biogeochemistry of oxygen minimum zones using qPCR to quantify absolute functional gene abundance (Reed *et al.*, 2014). In contrast, here we employ the approach in the context of a spatially (and temporally) resolved hydrothermal plume with the model directly driven by and validated with metagenomic data derived from samples taken in the plume and from the surrounding water column. The model integrates physico-biogeochemical processes associated with hydrothermal plumes into a coherent whole and is applied to a site in the Lau basin, a back-arc basin located in the west of the South Pacific Ocean. The overarching goal of the present study is to explain the structure of microbial communities within hydrothermal plumes, while identifying the environmental factors that govern the geochemical potential of these communities. First, we consider the role of physics in shaping the microbial community in a hydrothermal plume. Then, we introduce a subset of seven chemolithoautotrophic functional groups (that is, seven metabolic pathways) and demonstrate biogeochemical processes in action. Next, an additional pathway, hydrogen oxidation, is incorporated to explore a more complex scenario involving metabolic versatility. Finally, we use the results of these simulations to consider the broader impact of plumes on deep-sea biogeochemistry.

Materials and methods

Physico-biogeochemical model

A RTM of a hydrothermal plume was developed that considers the distributions and fates of chemical and biological species associated with various chemolithoautotrophic metabolisms. Microbial populations are modelled using a gene-centric approach, whereby organisms that mediate different metabolic pathways are tracked using abundances of associated functional genes (Reed *et al.*, 2014). For example, a state variable for the *amoA* gene represents the abundance of ammonia oxidising microbes. In addition, there are

also state variables for metabolic reactants (for example, oxygen and ammonia) and products (for example, nitrite). Each sub-population is associated with one metabolism with the exception of sulphur oxidation. The dominant sulphur oxidiser in the region, SUP05 (Sheik *et al.*, in press; K Anantharaman, personal communication), possesses all of the genes necessary to completely oxidise various reduced sulphur species to sulphate (Anantharaman *et al.*, 2013). Consequently, these metabolisms are combined into a single group. All metabolisms that are considered by the model are presented in Supplementary Table S1.

Within the RTM, microbial growth is described by means of Michaelis-Menten kinetics for both electron donors and acceptors (see Supplementary Material). Uptake of enzymatic substrates is linked to growth using an empirical relationship that expresses growth yield as a function of energy of the metabolism (Roden and Jin, 2011). Mortality is modelled using a first-order term for gene abundance. These biogeochemical processes—growth, death, consumption and production—are then cast into a physical transport framework. Parameters pertaining to these processes are given in Supplementary Table S2.

While the Roden and Jin empirical model (RJM, hereafter) that relates biomass yield and metabolic energy yield (that is, Roden and Jin, 2011) provides an invaluable first-order estimate of this relationship, there are a number of issues that must be borne in mind when employing this approach. First, the RJM is largely derived from organic metabolisms—with the exception of aerobic hydrogen oxidation—whereas we consider inorganic reactions here. Nevertheless, to our knowledge there is currently no equivalent model exclusively for inorganic metabolisms. Next, the model may overestimate biomass yield, as it derived from experiments performed under optimal conditions. Consequently, the model does not account for transient environmental conditions or other natural pressures. Also, since the studies used to derive the relationship did not typically provide detailed information about the concentrations of the reactants and products—data that are required to calculate metabolic energy yields—these concentrations were estimated from typical culture experiments. Finally, the model does not consider the impact of maintenance energy of biomass production, which could be potentially important in natural settings. Nevertheless, despite these caveats, the model remains a useful tool.

Hot fluid emanating from hydrothermal vents is less dense than seawater and thus ascends as it is injected into the cold water column, forming a plume as a result. Ambient seawater is entrained as the plume rises, reducing its buoyancy and vertical velocity until a neutral buoyancy is ultimately achieved (Helfrich and Speer, 1995; McDuff, 1995). Momentum carries rising plume water beyond this point, before gravity returns it to the depth of neutral buoyancy and

spreads laterally (Jiang and Breier, 2014). With this structure in mind, the model's physical domain is divided into two connected regions: the rising plume and the neutrally-buoyant plume.

The rising plume is spatially resolved along a 1D domain that is oriented vertically, parallel to flow (Figure 1). Rise velocity, turbulent mixing and the geometry of the rising plume are prescribed from an existing 2D axisymmetric fluid dynamics model (FDM; Jiang and Breier, 2014). In the RTM, the 1D rising plume domain is divided vertically into a series of adjacent, non-overlapping volumes. The height of these volumes is set by the node spacing along the centre axis of the plume from the FDM. The radius of each of these volumes—that is, the edge of the rising plume—is determined as the first point in the FDM where the lateral velocity is towards the centre of the plume (that is, where entrainment begins). The efflux of water at the top of each volume is typically greater than the influx at the base of the volume and, therefore, neighbouring seawater is entrained into the rising plume to conserve volume. In the RTM, rise velocities and turbulent mixing coefficients are an average of all FDM nodes that fall within each volume.

Parameters for the rising plume region of the RTM were generated by the FDM during simulation rke7_0 (Jiang and Breier, 2014), which represents a steady-state plume emanating from vent A1 located in the ABE hydrothermal field (20°45.672883' S 176°11.434418' W, depth 2140 m). Within the FDM,

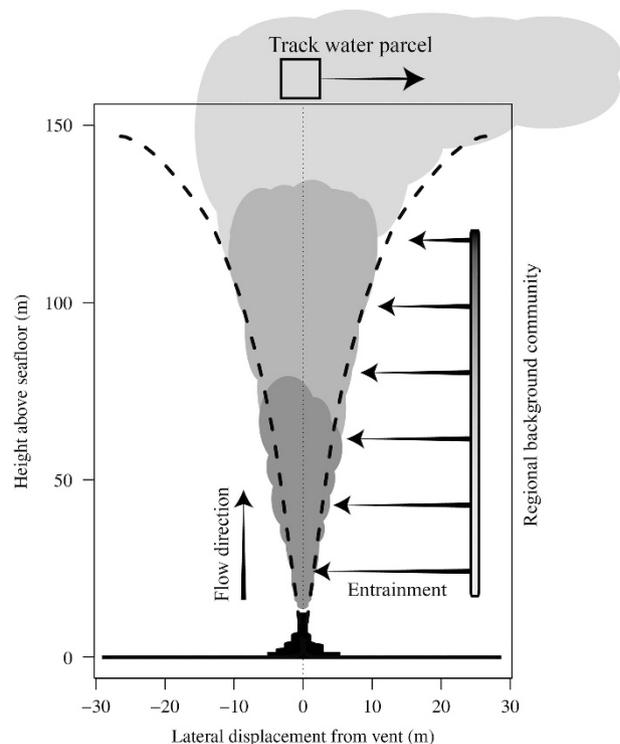


Figure 1 A schematic of the model domain showing the shape of the rising plume, entrainment of ambient seawater, and the export of a water parcel to the neutrally-buoyant plume.

a k - ϵ model is used to represent turbulence, and mixing coefficients for the RTM are calculated from the turbulent viscosities output from the FDM, assuming a turbulent Schmidt number of 0.7. For simulation rke7_0, ambient seawater temperature was prescribed as 2.2 °C at the base of the FDM domain and 2.3663 °C at the top of the domain (that is, 400 m above the seafloor), while ambient salinity was set as 34.65 resulting in a buoyancy frequency 0.000473 s^{-1} (Jiang and Breier, 2014). The chimney diameter and height were set at 0.14 m and 2 m, respectively, and hydrothermal fluid was expelled at 0.2 ms^{-1} , with a temperature of 309 °C. The resulting plume achieved a maximum height of 201 m. For thermodynamic calculations, the pressure is assumed to be a constant 210 bar.

Unlike the spatially resolved rising plume, the neutrally-buoyant plume is simulated in the RTM by tracking a unit volume of water exported from the rising plume over time as it spreads laterally. No additional dilution is assumed to occur in the neutrally-buoyant plume, because mixing is substantially lower than in the rising plume (McCollom, 2000), and we assume no feedback to the rising plume.

To run the model, values for the chemical and biological state variables must be prescribed at the vent orifice and in the surrounding seawater. The chemistry of nearby seawater is defined to represent 'typical' deep-sea water. Initial plume chemistry is prescribed using an existing reaction-path model (Breier *et al.*, 2012; Anantharaman *et al.*, 2014), which accounts for the rapid abiotic precipitation of minerals and chemical speciation in the vicinity of the chimney that results from the mixing of vent fluid (Mottl *et al.*, 2011; Flores *et al.*, 2012) and background seawater (Supplementary Table S3); details of this model for the A1 vent are described in Anantharaman *et al.* (2014).

Output of simulations describes the distribution of state variables over space (in the rising plume) and time (in the neutrally-buoyant plume). The RTM is coded in R (R Development Core Team, 2006) using the `tran.volume.1D` function of the `ReacTran` package to calculate transport terms (Soetaert and Meysman, 2012), while thermodynamic calculations are undertaken using the `CHNOSZ` package (Dick, 2008). Solutions are achieved by running the RTM to steady state in the rising plume using the `vode` numerical integrator from the `deSolve` package (Soetaert *et al.*, 2010). The model code is available at www.earth.lsa.umich.edu/geomicrobiology/Lau.html.

Metagenomic data

Microbial data used to drive and validate the model were collected from the ABE hydrothermal vent field, Lau basin, during cruises TN235 and TN236 aboard the R/V Thomas G Thompson in May–July 2009 (Supplementary Table S4; Anantharaman *et al.*, 2014). Sampling was undertaken using a

suspended-particle rosette multi-sampler (Breier *et al.*, 2009) with $0.8 \mu\text{m}$ filters. DNA extracted from $\frac{1}{4}$ of a filter, as described by Dick and Tebo (2010), was amplified using multiple displacement amplification before undergoing shotgun sequencing (Anantharaman *et al.*, 2014). Sequences can be accessed from the NCBI Sequence Read Archive using accession numbers SRX511269, SRX511304 and SRX511275 (see Supplementary Table S4).

Samples were collected by precision navigation using the remotely operated vehicle (ROV) *Jason* in dynamic positioning mode. *Jason's* position relative to the seafloor and target vent was measured in real time by long baseline acoustic navigation, supplementing the depth (pressure) sensor and altimeter on the vehicle. Using this navigation system, we found the orifice of our target vent, ABE, and sampled directly at the base of the plume, which was identified visually using the ROV. *Jason* was then directed to rise into the plume following its trajectory, which could be tracked visually using the backscatter from the vehicles laser pointers to 60 m above bottom. From there, the ROV then rose directly to the non-buoyant plume height of 200 m. Synoptic particulate samples were taken and the Al/(Al + Fe + Mn) ratio of these particles determined to confirm that sampling occurred within the plume, as reported by Sheik *et al.* (in press). The field methodology for this ROV-based plume sampling was described in Breier *et al.* (2009, 2014). The latter study includes a Supplementary video showing this approach.

Custom databases were created for functional genes associated with the modelled metabolic pathways (Supplementary Table S5). These databases were constructed using a combination of COG/Pfam-based functional annotations, identification of metabolic genes in the Lau metagenome (Anantharaman *et al.*, 2014), and reference genes identified from NCBI GenBank. These databases are available online at the following URL: www.earth.lsa.umich.edu/geomicrobiology/Lau.html. Raw shotgun sequencing reads were trimmed using the adaptive read trimmer, Sickle (<https://github.com/najoshi/sickle>). Trimming removed 0.14–2.8% of low quality reads. Trimmed forward and reverse reads from a single sample site were concatenated and BLASTX (Altschul *et al.*, 1990) was performed against the custom databases for individual genes using the following parameters: *e* value $< 1e-5$, alignment length $> 60 \text{ bp}$, bit score > 50 , percent identity > 60 . The resultant data were normalised for variance in data set size and gene length.

Results and discussion

Entrainment shapes rising plume communities

In the model, microbial populations and chemical species are tracked over a 1D domain representing the rising plume and then over time within the neutrally-buoyant plume (Figure 1). Figure 2 shows

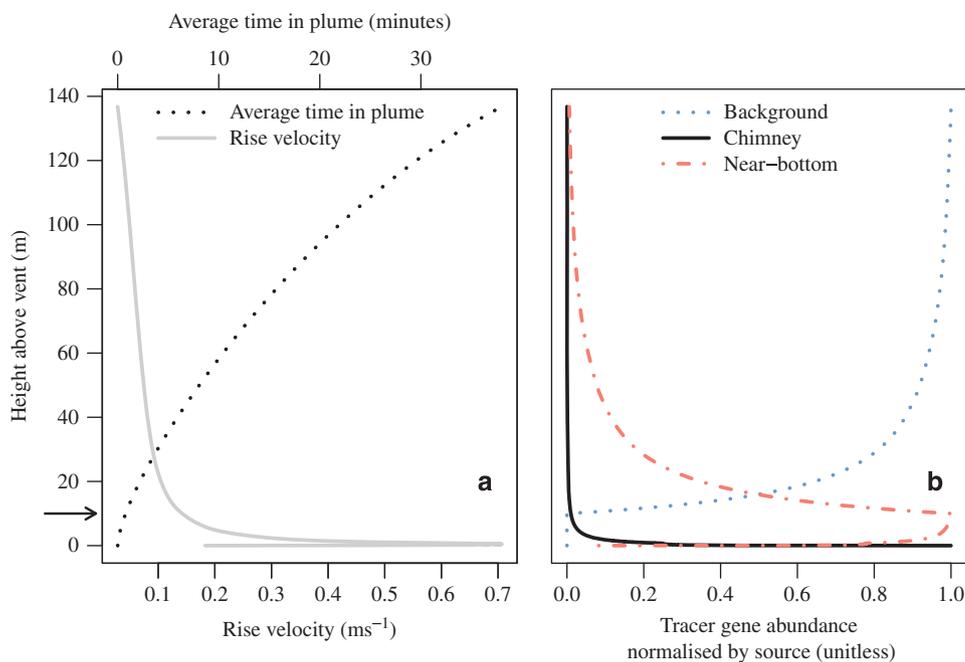


Figure 2 (a) Rise velocity (gray solid line) and average time that vent fluid has spent within the rising plume (black dotted line). (b) Distribution within the plume of inert tracers of microbial communities that originate from the chimney region (black solid line), demersal layer (pink dot-dashed line) and pelagic seawater (blue dotted line).

rise velocity and average time within the plume as a function of height above the orifice. Vent fluid initially accelerates after it is injected into the water column, as it is less dense than seawater and, therefore, more buoyant (Jiang and Breier, 2014). Seawater is quickly entrained and mixed with this vent fluid near the chimney, reducing its buoyancy and slowing the ascent of the plume (Converse *et al.*, 1984). As a result, the maximum rise velocity ($\sim 70 \text{ cm s}^{-1}$) is achieved just above the orifice. Maximum average time in the rising plume is less than 40 min, which is quite short relative to the doubling time of low-temperature, deep-sea chemolithoautotrophs (for example, Martens-Habben *et al.*, 2009) suggesting minimal growth in this region. Nevertheless, before considering the role that biological and geochemical factors play in shaping plume ecosystems, the model is used to examine the impact of physics.

As they rise, hydrothermal plumes entrain large quantities of seawater, which contain ambient microbial communities and chemical species. The initial plume community (comprised of microbes near the vent orifice) is thus diluted with neighbouring waters that bear potentially different microbial communities. To quantify the influence of entrainment on the structure of plume communities, a simulation was undertaken in which marker genes were used to track inert microbial populations (that is, no growth or mortality) from three regions: near chimney and vent fluid, ambient seawater, demersal (that is, near-bottom) layer. Results demonstrate that, in the absence of growth and mortality, microbes living upon or around chimney structures

that are drawn into the plume at the vent orifice are rapidly diluted to near zero abundances due to entrainment of seawater (Figure 2). These organisms may be thermophilic and would therefore be unable to grow appreciably in the rising plume, meaning their contribution to the microbial community is likely negligible above the initial $\sim 10 \text{ m}$ of the plume. This suggests that vent or chimney communities cannot be invoked as the source of elevated cell counts in rising plumes (for example, Winn *et al.*, 1986).

Demersal microbial communities, sustained by diffuse flow of reduced chemicals emanating from the broader vent region, are assumed to occupy a 10-m layer overlying the seafloor in the simulation presented in Figure 2 (Huber *et al.*, 2003). These microbes are quickly entrained into the plume and reach a maximum abundance at the upper edge of the layer. Beyond the demersal layer, their numbers decline due to entrainment of seawater, as seen previously for chimney communities, although at a slower rate. Indeed, demersal microbes are still found at appreciable abundances at 50 m height above the vent orifice. If these organisms are able to utilise substrates of vent origin at relatively low temperatures and low concentrations, then entrainment effectively primes the plume with the near-bottom community ready for growth. A potential example of this in nature is the gammaproteobacterium SUP05, which is found in diffuse flow regimes yet also thrives in hydrothermal plumes (Huber *et al.*, 2003; Sunamura *et al.*, 2004; Anderson *et al.*, 2012; Lesniewski *et al.*, 2012; Marshall and Morris, 2013).

At the uppermost point of the modelled rising plume, the ratio of vent fluid to ambient seawater is on the order of $1:10^4$, consistent with observations (for example, Lupton *et al.*, 1985). Thus, when considering physical processes alone, pelagic deep-sea microbes are shown to dominate the microbial community over the majority of the plume due to entrainment of ambient seawater (Figure 2). This is in agreement with previous studies of hydrothermal plumes (for example, Lesniewski *et al.*, 2012). Nevertheless, these communities are also influenced by biogeochemical processes, which depend on numerous factors, such as the availability of enzymatic substrates and nutrients, cell abundances and mortality. These processes are discussed below.

Predicting the functional composition of the microbial community

Incorporating microbial communities into the model requires that the abundances of each functional group are specified at the vent orifice and within the surrounding ambient seawater. These values are determined from observations using metagenomic data, which give the relative abundances of pertinent functional genes, and an assumed total abundance of 10^6 functional genes L^{-1} , reflecting deep-sea cell abundances on the order of 10^6 – 10^7 $cell\ l^{-1}$ (for example, Karner *et al.*, 2001). The abundance of microbes in ambient seawater is assumed to increase by 5.5% over the height of the plume at ABE (Karner *et al.*, 2001). Metagenomic data are also available for the microbial community within the neutrally-buoyant plume, at a height of 200 m above the vent orifice, which is used to validate model results through comparison with the community predicted by the model in this region.

Simulations undertaken with active microbial communities demonstrate that there is negligible growth in the rising plume and, therefore, the structure of the microbial community entering the neutrally-buoyant plume is very close to that of ambient seawater (results not shown). The majority of microbial growth thus occurs in the neutrally-buoyant plume, as shown in Figure 3, which follows the temporal development of the microbial community beyond the rising plume. Simulations show that model predictions and observations are in closest agreement after 132 h when all metabolisms are reproduced accurately (Figure 4). All metabolisms exhibit growth over time, although the rates of gene production depend on the availability of the associated electron donor and the energy yield of the reaction (Figure 3b). As a result of these differing rates, there is a marked shift in the relative abundances of genes over time (Figure 3a).

While sulphur oxidising microbes are abundant close to the vent orifice—as vent fluids are replete in sulphur species—dilution with seawater attenuates this population to background levels before marked growth occurs in the neutrally-buoyant plume

(Figure 3). Ammonia oxidising bacteria and archaea are abundant in ambient seawater, comprising $\sim 70\%$ of the microbes considered (Figure 4). Nevertheless, in spite of substantial entrainment of such seawater, they represent only around 10% of the observed population in the neutrally-buoyant plume due to marked growth of sulphur oxidisers. Most of the modelled populations exhibit an exponential growth phase followed by a quasi-plateau in absolute abundances when enzymatic substrate is exhausted. Ultimately, after reaching maximum abundances, these populations decline slowly over time due to mortality and an absence of growth (Figure 3a). The absolute abundance of nitrite oxidisers shows an upward curvature over time, however, because nitrite is produced within the plume (Figure 3b). As the ammonia oxidising community grows, the rate of nitrite production increases. Simultaneously, the population of nitrite oxidisers grows, which also enhances the rate of nitrite oxidation. Note that abundance of ammonia oxidisers is much higher than that of nitrite oxidisers, consistent with previous observations in the Guaymas Basin hydrothermal plume (Baker *et al.*, 2012). ABE is hosted in andesitic basalts and, therefore, vent fluid is relatively low in methane, in contrast to ultramafic systems (McCollom, 2007). This is reflected in the microbial community with few methanotrophs present (Figure 3b).

A Monte Carlo approach was employed to examine the impact of kinetic parameters on model output. Half-saturation constants and maximal growth rates for all metabolisms were varied randomly within $\pm 25\%$ of their original values over 50 simulations, assuming a uniform distribution across the $\pm 25\%$ interval, and the results were consolidated to determine the range of responses (grey region, Figure 3). A random parameter sampling approach was used, because relative abundances for each functional group depend not only upon their own growth parameters, but also on growth parameters for other metabolic pathways. While varying growth kinetics affects the specific trajectory of each population, the general trends remain the same since metabolic energy yield exerts a greater influence over the community structure (Figure 3).

Results presented here suggest that energy availability shapes microbial communities that inhabit hydrothermal systems, in agreement with previous studies (for example, Shock *et al.*, 1995; McCollom and Shock, 1997; McCollom, 2000, 2007; Amend *et al.*, 2011; Boettger *et al.*, 2013; Nakamura and Takai, 2014). Nevertheless, while this principle may be accurate in a broad sense, observations that do not conform to this paradigm hint at unresolved mechanisms and additional complexities. One such example is encountered when considering aerobic hydrogen oxidation and the abundance of associated hydrogenases. As shown for ammonia oxidation,

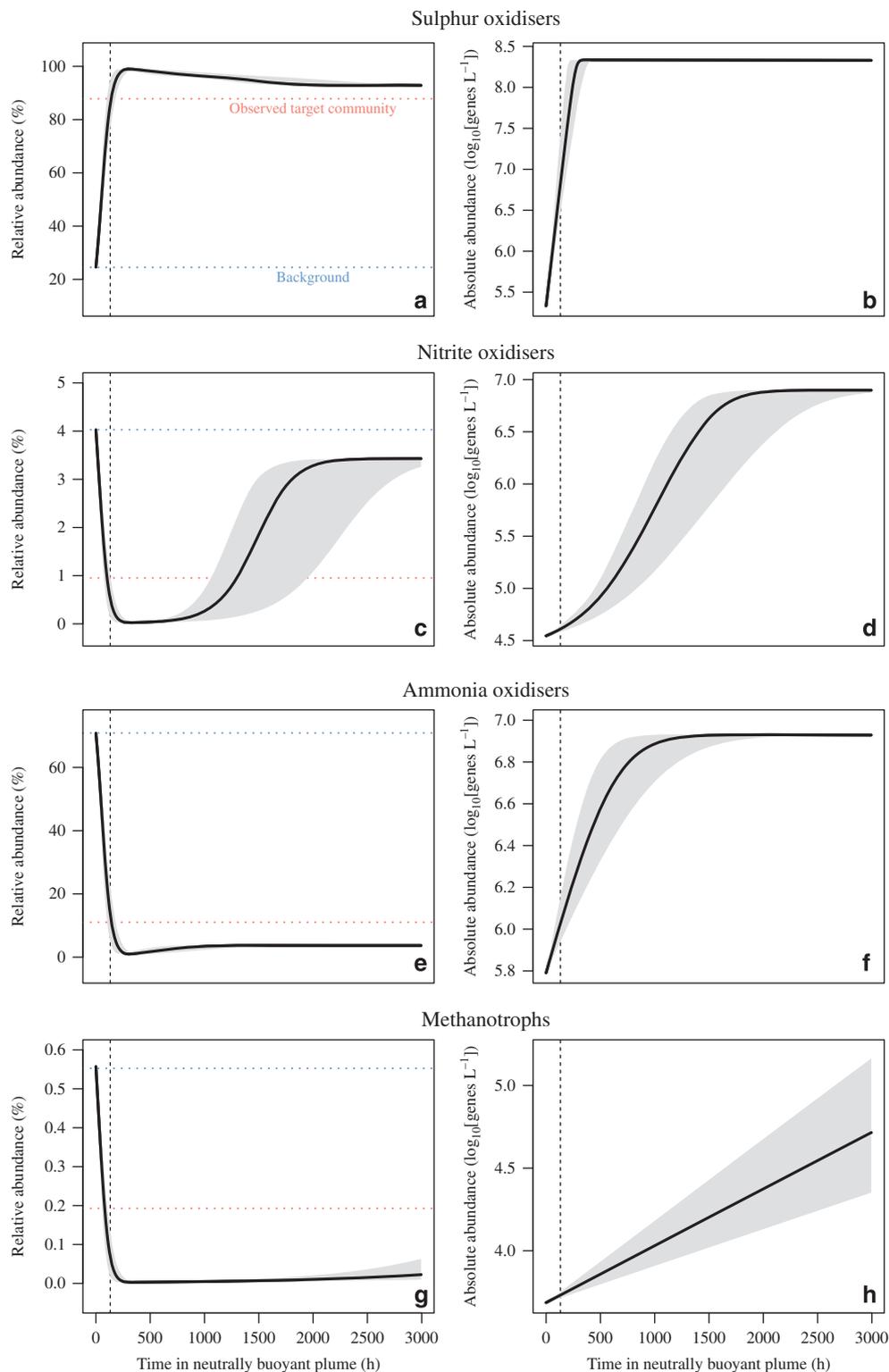


Figure 3 Relative and absolute abundances of functional genes for modelled microbial communities associated with seven different aerobic metabolisms versus time in the neutrally-buoyant plume. (Note that four pathways are consolidated into the sulphur oxidation pathway.) The solid black line shows the baseline scenario and the grey regions show range achieved from a Monte Carlo experiment using growth parameters selected randomly from within $\pm 25\%$ of baseline values assuming a uniform distribution of parameters. Vertical, black dotted line shows the point of closest agreement between observations and predictions. Pink dotted line shows the observed community within the neutrally-buoyant plume, while the blue dotted line shows the observed community of ambient seawater.

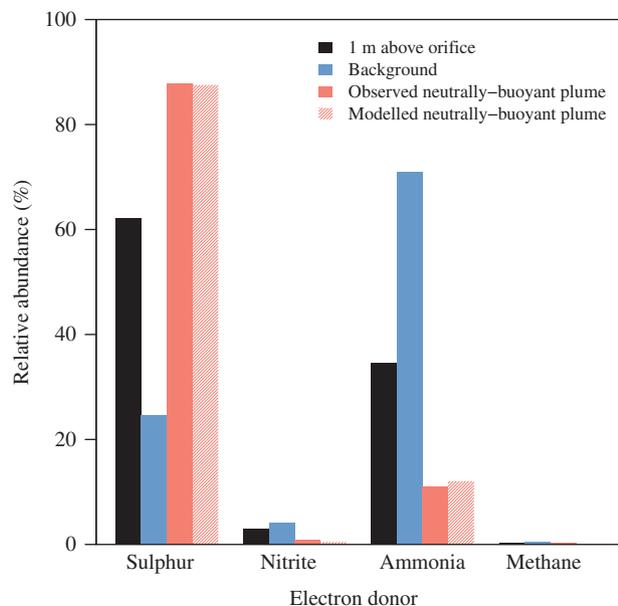


Figure 4 A comparison of observed (pink solid) and predicted (pink cross-hatched) relative abundances of functional genes after 132 h within the neutrally-buoyant plume (i.e., the moment of closest agreement). Also plotted are the relative abundances of functional genes in background seawater (blue) and at 1 m above vent orifice (black).

rapid growth of sulphur oxidising microbes overwhelms metabolisms that do not experience appreciable growth in the plume, even if they are abundant in the entrained background community (Figure 4). Vent fluids at ABE are depleted in hydrogen, as ABE is hosted in basaltic andesites, which precludes significant growth within the plume *via* hydrogen oxidation. One would therefore expect the relative abundance of hydrogenases to be attenuated below background levels in the plume; however, this is not the case (Figure 5). These observations are explored in detail below.

Insights from model-data discrepancies

Expanding upon the suite of metabolisms used above, aerobic hydrogen oxidation was added to the model (that is, the 'Knallgas' reaction, $\text{H}_2 + \frac{1}{2}\text{O}_2 \rightarrow \text{H}_2\text{O}$), as this has been identified as a potentially important pathway in other hydrothermal systems (for example, McCollom, 2000, 2007; Anantharaman *et al.*, 2013). Compared with those systems, however, fluid emanating from the ABE vent is somewhat depleted in H_2 (54–101 $\mu\text{mol kg}^{-1}$; Flores *et al.*, 2012) suggesting that the relative abundance of hydrogenases—genes associated with hydrogen oxidation—should also be low. Nevertheless, in spite of low hydrogen concentrations, substantial dilution, and marked growth by sulphur oxidisers, the relative abundance of hydrogenases remains above background levels (Figure 5). Consequently, assuming that hydrogenase genes are primarily produced using energy derived from hydrogen oxidation leads to discrepancies between

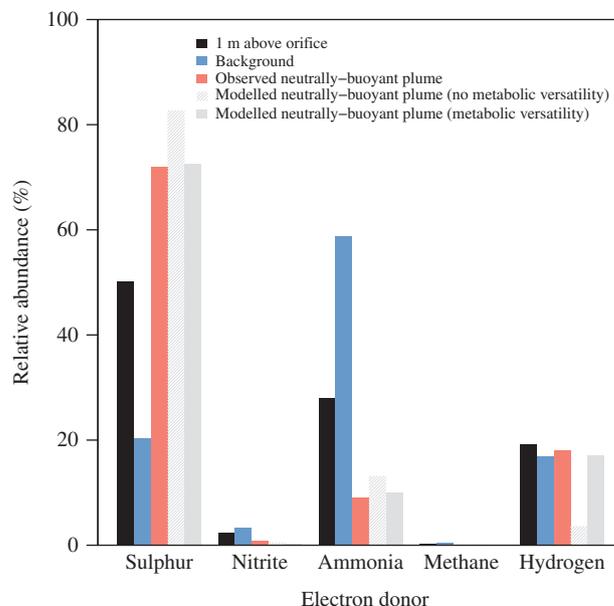


Figure 5 A comparison of observed (pink solid) and predicted (grey solid and cross-hatched) relative abundances of functional genes after 132 h within the neutrally-buoyant plume (i.e., the moment of closest agreement). The cross-hatched grey bar shows simulation results without metabolic versatility (i.e., no hydrogenase production by sulphur oxidisers), while the solid grey bar shows results from a simulation with metabolic versatility implemented. Also plotted are the relative abundances of functional genes in background seawater (blue) and at 1 m above the vent orifice (black).

model predictions and observations. These disagreements suggest that important mechanisms for hydrogenase production have been omitted from the model.

A recent metagenomic analysis of the vent fields in the Lau basin revealed that sulphur oxidisers are the most abundant chemolithoautotrophs and that SUP05 dominates this metabolism (K Anantharaman, personal communication; Sheik *et al.*, in press). Some populations of SUP05 are metabolically versatile, possessing genes for all sulphur oxidation pathways (hydrogen sulphide, thiosulphate, elemental sulphur, sulphite), as well as hydrogenases (Anantharaman *et al.*, 2013). Given the prevalence of both sulphur oxidation and SUP05 in the region, one would expect these organisms to contribute towards the abundance of hydrogenases. Assuming that SUP05 constitutes ~20% of the plume's sulphur oxidising community and, therefore, contributes towards hydrogenase production, generates model predictions that are in close agreement with observations (Figure 5). Metagenomic analysis reveals that the sulphur oxidising community in ambient seawater is 10% SUP05 and 27% SUP05 at 1 m above the vent, which presumably reflects the near-vent demersal community (for example, from the diffuse flow region, biofilms on chimney structures) because vent fluids are too hot to host a native microbial community. Given that the plume community is a mixture of demersal and

pelagic communities, these data suggest that assuming the sulphur oxidising community is 20% SUP05 is quite reasonable. In this scenario, sulphur oxidation accounts for the production of the majority of hydrogenases. Nevertheless, the model overestimates the abundance of sulphur oxidation genes if the SUP05 sub-population is assumed to grow by means of aerobic hydrogen oxidation (results not shown), which is discussed in detail below.

There are several possible factors that may contribute towards inappreciable growth *via* the Knallgas pathway. Hydrogen uptake in low concentration environments, such as at vent A1 in the ABE field, progresses at a much slower rate than in environments where H₂ is abundant (Petersen *et al.*, 2011). Therefore, spontaneous abiotic oxidation of H₂—which occurs gradually over several hours—may deplete hydrogen before appreciable microbial growth *via* the Knallgas metabolism is able to occur (McCollom, 2000). Furthermore, H₂ is also consumed as an electron donor for other metabolisms, such as sulphate reduction, methanogenesis and iron (III) reduction (McCollom, 2000). Indeed, while there are microbes that are able to grow by means of aerobic hydrogen oxidation, these organisms are typically capable of other metabolisms as well (for example, sulphur oxidation, methanotrophy; Petersen *et al.*, 2011; Anantharaman *et al.*, 2013). Metagenomic analysis of the microbial community in the vicinity of the ABE vent field has revealed no organism that subsists exclusively *via* the Knallgas metabolism (K Anantharaman, personal communication).

These simulations highlight the difficulties in inferring the occurrence of metabolisms from the presence of functional genes alone without corresponding transcriptomic or proteomic data, which could indicate which metabolisms are actually active. Nevertheless, while the Knallgas pathway may not contribute appreciably to microbial growth at vent A1, an abundance of hydrogenases mean that the community has the potential to mediate this metabolism should a hydrogen-rich environment be encountered. Metabolic versatility of this sort is likely a key ecological strategy employed by organisms that rely upon electron donors that occur in low concentrations in the deep ocean. Specifically, the propagation of metabolic genes due to growth *via* other pathways retains the ability to respond to different energy sources by means of a bet-hedging approach.

Impacts of hydrothermal perturbations beyond the rising plume

Electron donors that are exported from rising plumes and dispersed *via* physical processes represent a potential source of energy for deep-sea chemolithoautotrophs. Growth of these microbes is not only controlled by the availability of electron donors, however, but also by the energy yield of the reaction that is mediated. Multiplying the electron

donor concentration at the top of the rising plume (mol l⁻¹) by the rate of plume water export (l s⁻¹) and energy yield per mole of electron donor (kJ mol⁻¹) gives an estimate of energy export per unit time (kJ s⁻¹), which is subsequently converted into an export of potential biomass *via* an empirical relationship (Roden and Jin, 2011), assuming these species are aerobically oxidised.

Figure 6 shows this export of potential biomass as a function of metabolic energy yield and electron donor concentration at the apex of the rising plume, assuming that all of the electron donor is used for growth. This represents an upper bound, as in nature a portion of the electron donor will be unavailable when concentrations become low. Moreover, abiotic reactions or other metabolisms not considered here may also consume the electron donor. Nevertheless, this figure allows the potential biomass production for these metabolisms to be compared relative to one another, as well as providing an upper limit on biomass. Highlighted within the plot are the metabolic pathways that are modelled above for vent A1 in the ABE field. Sulphur is shown to dominate since it is found in relatively high concentrations and is energetically profitable. In contrast, while methanotrophy yields a greater amount of energy per mole of electron donor, it is found in low concentrations meaning that it is but a minor player. Aerobic oxidation of hydrogen, ammonia and nitrite are all less energetically favourable and occur at low concentrations resulting in modest potential biomasses. Like potential biomass, the rate of growth is a function of both metabolic energy yield and enzymatic substrate availability. Consequently, metabolisms with a higher energy yield and substrate availability also grow more rapidly, which results in a temporal

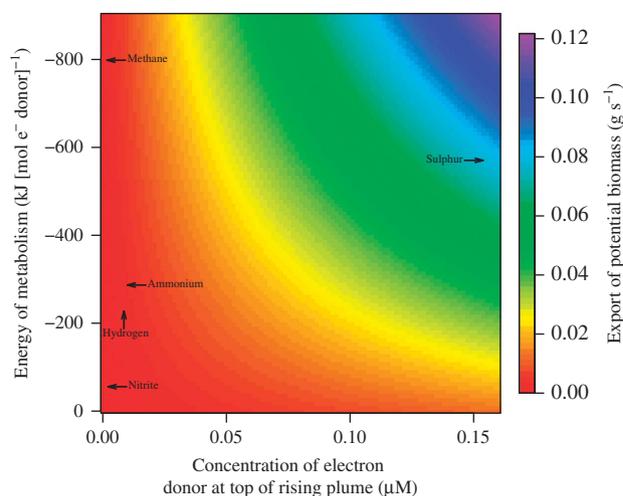


Figure 6 Export of potential biomass from the rising plume as a function of electron donor concentration and metabolic energy yield. Aerobic oxidation of ammonia, hydrogen, nitrite, methane and sulphur species for vent A1 of the ABE are plotted explicitly in the figure, assuming all of the electron donor is consumed by the considered reaction.

succession in the microbial community on the scale of weeks, as observed previously in hydrothermal plumes (Sylvan *et al.*, 2012). When combined with transport processes, this translates into a spatial succession with maximum abundances closer to the plume source for metabolisms with higher potential biomasses, as shown below.

Data derived from drifter experiments in the Lau basin allow the various time scales of microbial growth to be cast in a spatial context. Eleven autonomous APEX and PROVOR floats were deployed in the region between April 2004 and June 2005 (Speer and Thurnherr, 2012). These autonomous drifters maintained a depth of 1700 m—comparable to the depth of neutrally-buoyant hydrothermal plumes—and surfaced every 3–4 weeks to transmit data before returning to depth again. Tracking the displacement of numerous drifters relative to their point of deployment allows probability density functions to be constructed that quantify the likelihood a water parcel is found a given distance from its source (for example, a hydrothermal plume). When divided by drift times, these distances are translated into average speeds. Finally, multiplying these speeds by a time scale of growth determines how microbial communities in the neutrally-buoyant plume have been redistributed during this period of growth. The time scale used here is doubling time as calculated by the RTM, as it provides an intuitive measure of microbial growth.

Figure 7 illustrates how microbes associated with different metabolisms have been transported away from the hydrothermal vent over their doubling time. Since sulphur oxidation occurs relatively rapidly, little dispersion has occurred by the time the associated community has doubled in size, resulting in high abundances that are concentrated close to the vent. These metabolisms also generate the greatest biomass, which contributes further to dense concentrations of sulphur oxidisers close to the vent. In stark contrast, methanotrophy is relatively slow and, therefore, methane oxidisers undergo marked dispersion while growing. In addition, methane oxidisers produce less biomass than the other metabolisms considered. Thus, while methanotroph abundances increase by an order of magnitude due to hydrothermal stimulation, slow growth rates relative to transport processes and lower biomasses relative to other microbes may mask this somewhat. Furthermore, these microbes are likely to continue grow much farther afield than sulphur oxidisers or other rapidly growing organisms.

Spatial succession due to microbial growth on different time scales is demonstrated here by tracking a water parcel along a specific trajectory. Figure 8 plots the paths of two different drifters, which were deployed close to the ABE vent field, over time and shows the predicted compositions of the microbial community at various points. Due to rapid growth and a high potential biomass, sulphur oxidisers quickly dominate the community with ammonia oxidisers accounting for the majority of

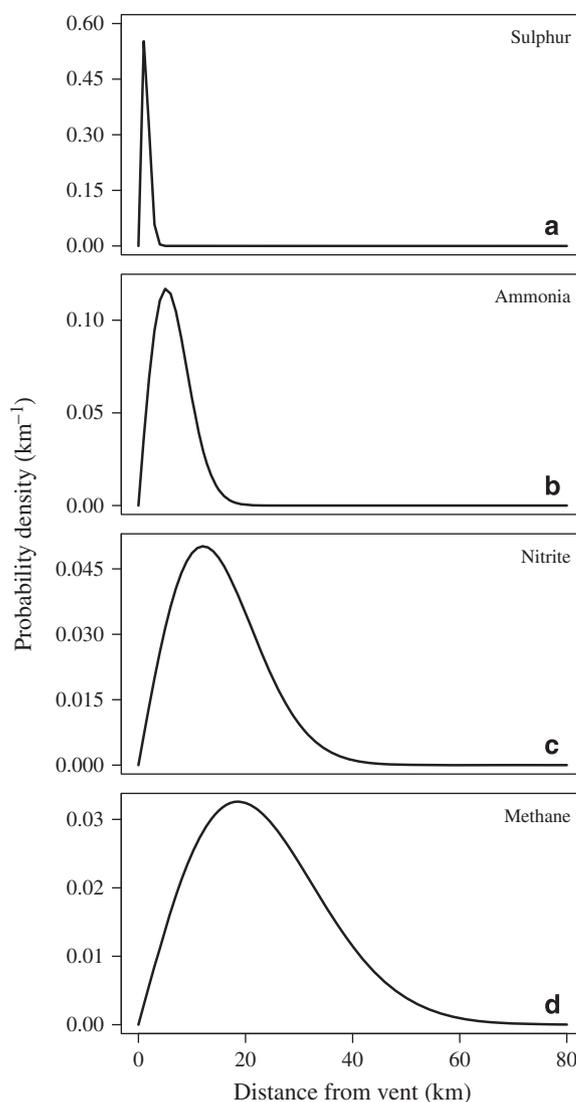


Figure 7 Probability density functions showing distance of water parcels from their source (i.e., the plume) as a function of the doubling time of each metabolism.

other organisms present. Nevertheless, as time progresses, microbes that mediate slower metabolisms begin to appear once again, first nitrite oxidisers and then methanotrophs, albeit at modest levels due to an abundance of sulphur oxidisers persisting due to the high vent fluid concentrations and energy yields (Figure 6).

While the spatial scales shown in Figures 7 and 8 correspond to growth, microbes may endure over much larger distances depending on mortality pressures, which are notoriously difficult to estimate. The mortality rate constant used here, 0.001d^{-1} , was constrained for deep-sea chemolithoautotrophs by a previous study (Reed *et al.*, 2014) and corresponds to a time scale that is much larger than those of growth (that is, 1 to nearly 3 orders of magnitude greater). These simulations point to a paradigm of microbial growth within tens of kilometers of the vent field, followed by marked

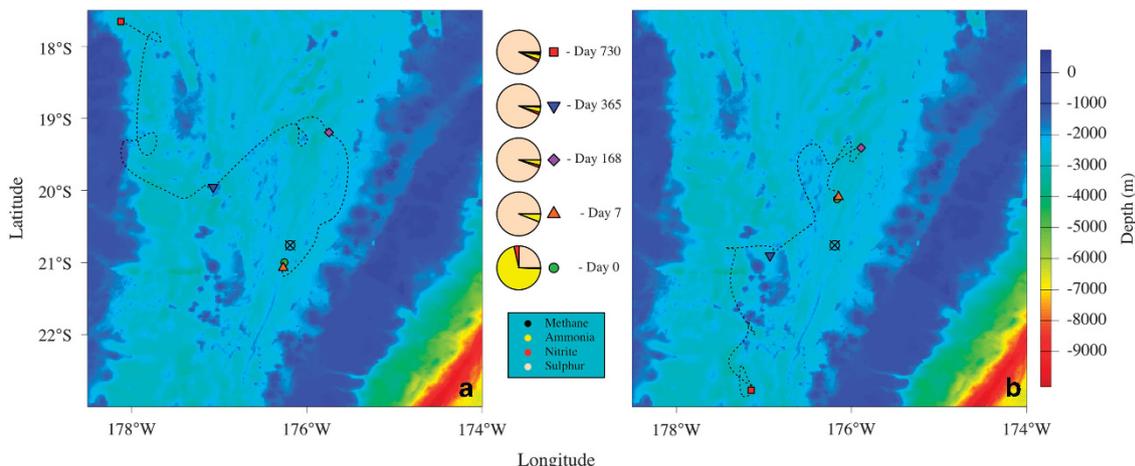


Figure 8 Modelled microbial communities are projected onto the trajectories (dotted lines) of two drifters released in the vicinity of the ABE vent field (denoted by a circle and cross marker). Symbols are plotted on each trajectory showing the location of the drifters at 0, 7, 168, 365 and 730 days. Corresponding to these times, pie charts show the structure of the microbial community. Background colours reflect the bathymetry of the region.

persistence of microbial communities over hundreds of kilometers. As the average distance between high-temperature hydrothermal systems is <100 km (German *et al.*, 2010), these results suggest that plumes have an important role in linking these systems. Furthermore, given the widespread distribution of hydrothermal vents along mid-ocean ridges, hydrothermal plumes potentially have a broad impact on deep-ocean biogeochemistry and microbial diversity by exchanging material with different marine systems that are hospitable to the similar microorganisms, such as oxygen minimum zones and oil spills (Dick *et al.*, 2013). However, given that plume communities are sourced from ambient seawater and become mixed with these ambient microbial communities over time, it is difficult to delineate these two communities. Indeed, feedback between plume and ambient communities may prove an important driver of dark-ocean microbial ecology.

Conflict of Interest

The authors declare no conflict of interests.

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Archive, accession numbers SRX511269, SRX511304 and SRX511275 (Supplementary Table S4). These data have been previously published by Anantharaman *et al.* (2014). The custom databases, blastx results and reads that hit each gene referenced in this study are available at the following URL along with the model code: www.earth.lsa.umich.edu/geomicrobiology/Lau.html

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