Feast and famine — microbial life in the deep-sea bed

Bo Barker Jørgensen and Antje Boetius*‡*

Abstract | The seabed is a diverse environment that ranges from the desert-like deep seafloor to the rich oases that are present at seeps, vents, and food falls such as whales, wood or kelp. As well as the sedimentation of organic material from above, geological processes transport chemical energy — hydrogen, methane, hydrogen sulphide and iron — to the seafloor from the subsurface below, which provides a significant proportion of the deep-sea energy. At the sites on the seafloor where chemical energy is delivered, rich and diverse microbial communities thrive. However, most subsurface microorganisms live in conditions of extreme energy limitation, with mean generation times of up to thousands of years. Even in the most remote subsurface habitats, temperature rather than energy seems to set the ultimate limit for life, and in the deep biosphere, where energy is most depleted, life might even be based on the cleavage of water by natural radioisotopes. Here, we review microbial biodiversity and function in these intriguing environments.

Benthic
Relating to or occurring at the seafloor.

Mud volcano
A large seabed formation (hundreds of metres to kilometres in diameter) that is caused by the eruption of subsurface gas, fluid and mud.

**Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, Bremen D-28359, Germany.*
‡Jacobs University Bremen, Campus Ring 1, Bremen D-28759, Germany.
Correspondence to B.B.J.
e-mail:
bjoergen@mpi-bremen.de
doi:10.1038/nrmicro1745
Published online
10 September 2007

Fifty years ago, the seabed was considered to be flat, uniform and biologically inert owing to the remoteness of its location from photosynthetic ecosystems and the extreme conditions that are present at the seafloor, such as high hydrostatic pressures (up to 1,100 bar) and low temperatures (less than 4°C). In the 1950s, sediment samples that were gathered on the Danish *Galathea* Deep-Sea Expedition from depths of more than 10,000 metres were shown to contain millions of viable bacteria per gram. The authors concluded that pressures of up to 1,000 bar are not a deterrent to bacterial life[1]. Later, the Deep-Sea Drilling Project in 1968 recovered highly diverse core material from subsurface sediments and the ocean crust, which precipitated a new perception of the ocean floor as a dynamic environment. The biological implications of this new viewpoint became strikingly clear with the discovery of hydrothermal vents in 1977. The famous dive of the deep-sea submersible ALVIN to the Pacific mid-oceanic ridge near the Galapagos Islands revealed amazingly rich benthic communities of giant clams, tube worms and microbial biofilms that were associated with the venting of hot fluids, temperatures of up to 370°C and emanating black smoke from chimneys that were composed of precipitated minerals[2].

Since then, the development of advanced instrumentation for observing, mapping and sampling the seabed and all of its features has enabled scientists to develop a picture of the deep-sea environment as a highly dynamic geo- and biosphere. Besides the vast desert-like plains of deep-sea mud there are also diverse landscapes of canyons, cold seeps, deep-water coral reefs, mud volcanoes, carbonate mounds, brine pools, gas hydrates, seamounts, ridges, fractures and trenches that are host to rich microbial communities, which we are only beginning to map and explore (BOX 1; FIG. 1).

This Review describes how the broad range of important benthic habitats in the deep sea provide highly diverse living conditions for microbial communities. Through their enormous catalytic potential and ability to couple multiple redox reactions of organic or inorganic compounds, the microorganisms can in turn modify these environments and affect seabed geochemistry on a global scale. Examples of the main deep-sea benthic environments, together with the microorganisms that inhabit them and the current approaches to their study, are described.

The deep seafloor

Almost 95% of the seabed (67% of the Earth's surface) lies in water depths where the light intensity is too low to sustain photosynthetic production, the temperatures are close to freezing (–1°C to 4°C) and the availability of organic matter controls benthic productivity and biomass. Depending on the productivity of the overlying waters, oxygen can be completely consumed within the

Anoxic
The absence of oxygen in the sea.

upper metre of sediments[3]. This top-metre layer of the seabed, which is mixed by burrowing animals, is defined as the ocean floor for this Review. For hard grounds, such as those found at the mid-oceanic ridges and in the central Pacific, it is more difficult to define the extension of surface (ocean floor) versus subsurface seabed, as the exchange with ocean waters is dependent on the porosity of the rock.

In the ocean floor, the order of depletion of microbial electron acceptors is usually oxygen first, then nitrate, oxidized manganese and iron minerals, then sulphate and finally bicarbonate[4]. Anoxic ocean floors — in which sulphate and carbon dioxide are the main electron acceptors — are found in upwelling areas and oxygen-minimum zones that are present at continental margins, as well as at hydrothermal vents and cold seeps. The most oligotrophic seafloor environments are found in the central ocean gyres, particularly in the South Pacific, where oxygen fully penetrates the thin sediment cover. Two mysteries have always fascinated deep-sea researchers who are investigating life at the deep seafloor: how do benthic communities satisfy their metabolic needs and which factors account for the high biodiversity of deep-sea benthic communities, particularly in the small size classes of organisms.

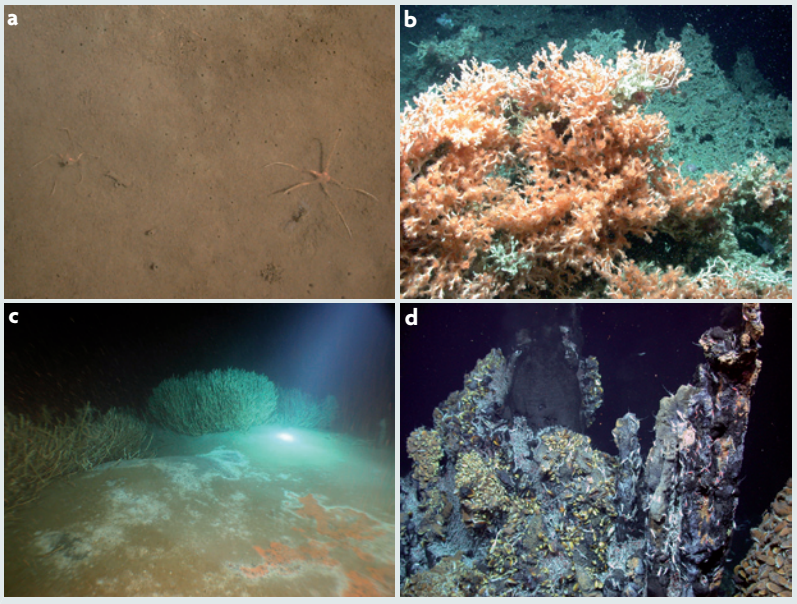
Most of the 320×10^6 km^2 of deep-ocean seafloor is covered with fine-grained sediments that are composed of biogenic, terrigenous, vulcanogenic and authigenic particles. Therefore, compared with the water column, the ocean seafloor provides a huge area of solid surfaces and heterogeneous pore spaces, as well as a high concentration of detrital organic matter per unit volume. In the sedimentary seafloor, transport processes, such as advection and mixing, are normally limited to a few centimetres per year by sediment porosity and compaction. Benthic microbial populations are partially immobilized so that distinct energy-rich gradients can form. Seafloor sediments contain 10–10,000-fold more cells per unit volume than productive ocean-surface waters (FIG. 2). This striking difference in microbial biomass between benthic and pelagic habitats is probably due to a combination of factors, such as the high energy availability at the seafloor and limited grazing pressure. Nevertheless, the largest part of the ocean seafloor receives little organic carbon from sedimentation — on average, 1 g of carbon per m^2 per year (REF. 5). Assuming that there is an average biomass of 10^8 cells per cm^3 in the top metre of sediment and a carbon mass of 20 fg per cell, this input has to nourish a standing stock of 2 g of carbon microbial biomass per m^3. Hence, even at an assimilation efficiency of 10%, and in the absence of other detritus feeders, the average turnover of the microbial community based on fresh organic matter input must be less than 5% per year at the seafloor and considerably less in subsurface sediments. A much larger potential source of organic carbon for energy and growth could be the aged, degraded detritus material that is buried in the sediments, which comprises approximately 0.1–1% of the sediment by dry weight. This sedimentary particulate organic matter (POM) is the equivalent of approximately 1–10 kg of carbon per m^3 at the seafloor, but is apparently not accessible for microbial degradation[6,7].

Box 1 | Geological formation of the seabed — setting the scene

The habitats of deep-sea benthic microorganisms are shaped by small- and large-scale geological processes. Solid plates of ocean crust and continents move around on the surface of our planet at speeds ranging from 1 to 10 cm per year, and dynamic sea-scapes are created where they diverge or collide (FIG. 1). New basaltic crust forms along the mid-oceanic ridges, where magma from the hot mantle convects up to the surface. Hot fluids are expelled at temperatures of up to 350–400°C[51] and mountain chains are created at depths of 2,000–4,000 m below the ocean surface. As the ocean crust slowly moves away, sinking particulate debris from the water column piles up to form sediment. In the central ocean, aeolian dust or minute carbonate skeletons of plankton organisms constitute an important fraction of the sediment, whereas material that is derived directly from the weathering of continents predominates in sediments that are closer to landmasses. The seabed functions as a giant conveyor belt, moving an ever-increasing sediment load away from the mid-oceanic ridges to the subduction zones, where this sediment is pushed back down into the hot mantle. When deposited sediment becomes compressed and geothermally altered, subsurface pore fluids and gases are expelled, forming gas-hydrate deposits, gas chimneys, mud volcanoes and diverse seep systems.

Some benthic habitats are shown in the figure, including: the bathyal ocean floor at a continental margin (**a**); a deep-water coral reef above a carbonate mound (**b**); tubeworm bushes and microbial mats at a cold seep (**c**); and a black smoker at a mid-oceanic ridge (**d**). Such geosystems are also abundant at passive margins, for example, those surrounding the Atlantic Ocean, where the continental plate moves with the ocean crust and thick sediment deposits build up, particularly in river fans. The total depth of deposited sediment ranges from metres in the central oceans to several kilometres at the continental margins, where sedimentation rates can be 100–1000-fold higher. As the earliest known ocean crust is more than 150 million-years old, it is possible to sample sediment records that date from modern time back to the Tertiary, Cretaceous and even Jurassic periods of the Earth's history.

In this Review, we define the seafloor as the top metre layer of the seabed that is bioturbated by animals, and porous ocean crust that is penetrated by seawater, whereas the deep subsurface, which harbours the deep biosphere, comprises the sediment and rock that is deeper than 1 metre beneath the seafloor. Image **a** is courtesy of the Monterey Bay Aquarium Research Institute, California, USA, image **b** is courtesy of IFREMER (French Research Institute for Exploitation of the Sea), Brest, France, image **c** is courtesy of I. MacDonald, Texas A&M University, Corpus Christi, USA and image **d** is courtesy of the MARUM Research Centre Ocean Margin, Bremen, Germany.



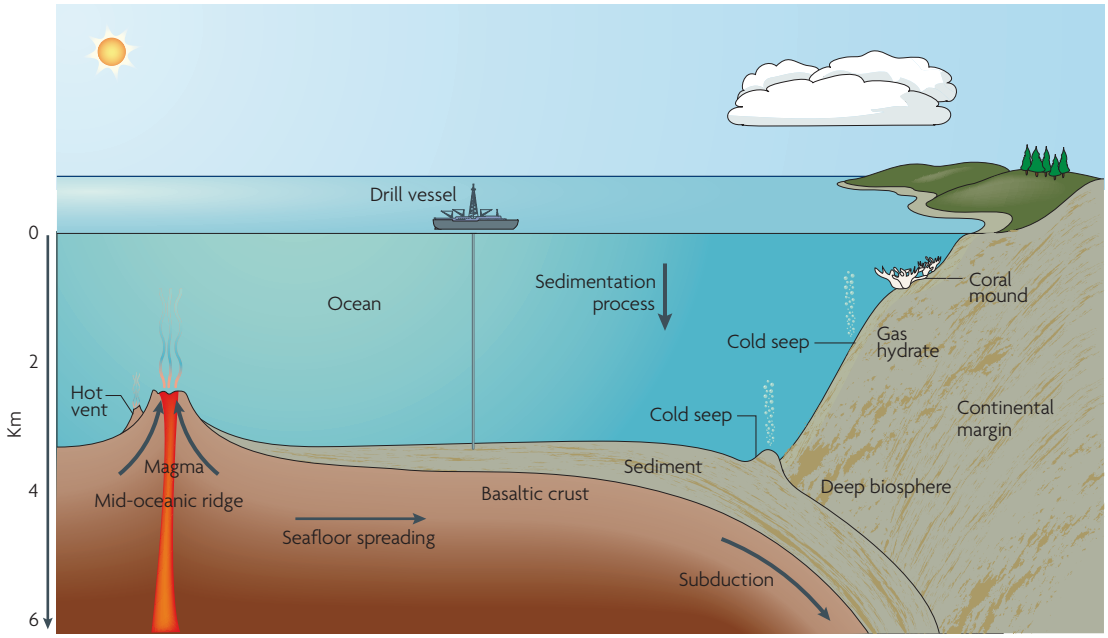


Figure 1 | **Vertical section of the seabed and seafloor structures.** This figure shows the plate-tectonic conveyer belt. At the mid-oceanic ridge new basaltic crust is formed continuously. The ocean plates move towards the continents where they are subducted again. Sinking particles form thick sediment piles on the ageing crust and on the continental margins. Plate tectonics and gravitational and hydrological forces cause a range of structures to form on the seabed, including hydrothermal vents at mid-oceanic ridges and subduction zones, gas-seeping mud volcanoes on continental margins and carbonate mounds inhabited by cold-water corals.

Oligotrophic
An aquatic environment that has low levels of nutrients and algal photosynthetic production (for example, high mountain lakes or the open ocean).

Terrigenous
Material that is derived from the terrestrial environment.

Detrital
Dead organic material.

Pelagic
Relating to or occurring in the water column.

Abyssal
Related to the deep seafloor (or abyss) that is situated between the continental rise (<3,000 metres) and the deep trenches (>6,000 metres), at an average depth of 4,000 metres.

Phytodetritus
The remains of dead plants, particularly of microalgae that originated from the surface waters.

Bioturbation
The displacement and mixing of sediment particles by benthic fauna (animals).

Humic substance
A degraded and chemically altered organic material.

Heterotrophic
The acquisition of metabolic energy by the consumption of living or dead organic matter.

Microbial abundance and degradation of organic matter at the seafloor. The first investigations of global trends in the distribution and abundance of microbial cells showed that the abundance of cells in surface sediments is usually related to the input of fresh detritus, rather than to any other oceanographic parameter[8–10]. Deming and Yager[11] were the first to show a global decrease in cell numbers along with an increase in water depth, based on approximately 1,000 samples from the surface of the seafloor (the top 0–1 cm layer). This was explained by the decrease in the particulate organic carbon (POC) flux that occurs with an increase in ocean depth and distance from land. A further analysis of six sample sites for which bacterial biomass and vertical POC flux was available showed a linear relationship between these parameters (FIG. 2). Lochte[12] found that the bacterial biomass in the top 10 cm of sediment increased twofold in response to the sedimentation of a spring plankton bloom in the abyssal northeast Atlantic. Approximately 1–2% of sedimented phytodetritus carbon is oxidized by benthic microorganisms within a few days, but re-mineralization rates slow down as the deposited material ages[13,14]. Feeding and bioturbation by macrofauna have an important role in POC degradation[15,16]. The input of phytodetritus and other labile organic materials to deep-sea sediments triggers the production of hydrolytic enzymes and, subsequently, the growth of the community, on similar timescales to those found in shallow-water sediments[17,18]. However, this labile organic matter is only a minor fraction of the aged and buried sedimentary organic material.

It remains poorly understood which fractions of the sedimentary organic material are accessible to microbial degradation and on what timescale these fractions are digested, oxidized and assimilated. Humic substances might be resistant to hydrolytic enzymes, and their use as an energy and nutrient source seems to depend on a range of physico–chemical and biological factors, such as the adsorption of organic matter to minerals or pre-digestion by animals[19]. There might be unknown mechanisms by which microorganisms desorb organic matter from mineral particles, and these might also have a role in the weathering of minerals in the seabed. The identification of the main microorganisms that are present at the seafloor and the enzymes that they use for organic matter hydrolysis and fermentation is necessary to gain an understanding of the degradation and burial of POM in the seabed.

Diversity at the seafloor. The general patterns in the distribution, diversity and dominance of functional and taxonomic groups of bacteria and archaea at the vast ocean seafloor are largely unknown. Considerable effort was required to isolate and cultivate certain barophilic and psychrophilic bacteria, such as some members of the *Moritella, Colwellia* and *Shewanella* clades[20]. However, their environmental relevance remains undetermined. Vetriani and colleagues[21] identified 16S ribosomal RNA (rRNA) genes of diverse uncultivated Crenarchaeota and Euryarchaeota spp. in the top 20 cm of deep-sea sediments. In the extensive sub-seafloor study by Inagaki and colleagues[22], bacterial members of the heterotrophic Chloroflexi and

Hot-spot ecosystem
An ecosystem of high or special biodiversity within a larger area of low or normal biodiversity.

Chemolithoautotrophic
The metabolism of an organism that obtains its energy from the oxidation of inorganic compounds and uses only carbon dioxide as a source of carbon.

archaeal members of the uncultivated miscellaneous Crenarcheota group dominated clone libraries from the top metre of sediment cores. Although thorough studies have not yet been done, in terms of cell biomass, archaea seem to comprise a small portion of the total microbial community of the detritus-fuelled oxic seafloor, in contrast with the anoxic subsurface, where archaea might dominate[23]. Possible shifts in benthic microbial community structure that could be associated with depth, latitude, season, composition of benthic fauna or POC fluxes have not yet been investigated, although such shifts are evident in the community structure of benthic animals. Furthermore, little is known about the roles, host specificity or diversity of the viruses in the seabed, although their numbers and production rates indicate that they might have a significant influence on bacterial mortality[24]. Most of the information on benthic microbial diversity has come from analyses of hot-spot ecosystems, such as hydrothermal vents, cold seeps and gas-hydrate systems.

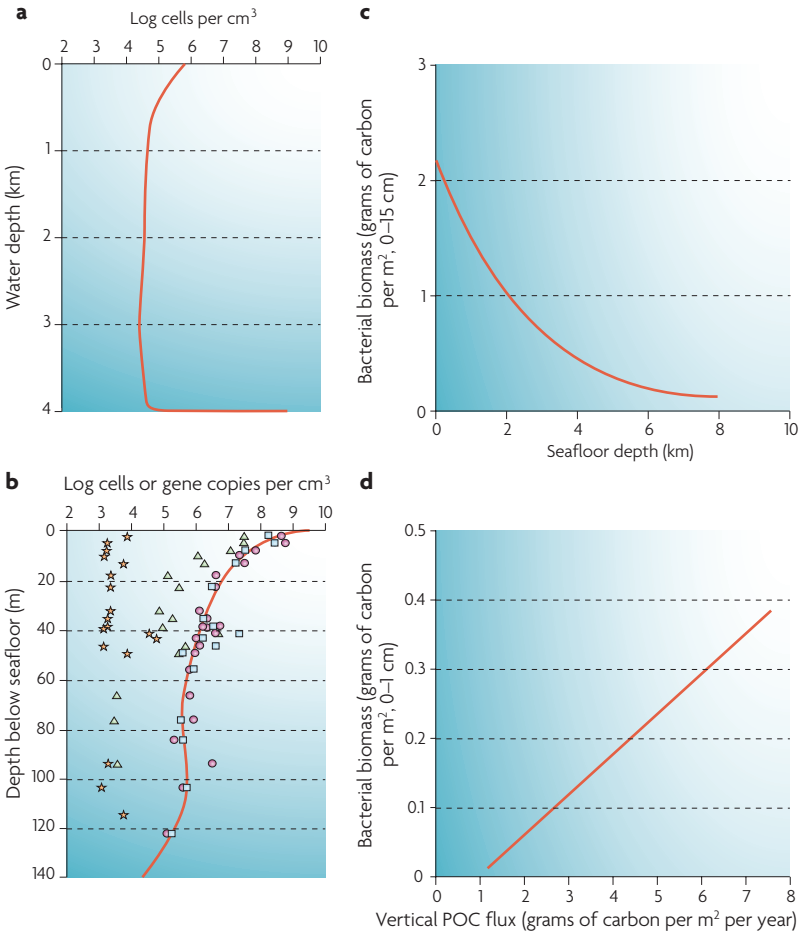


Figure 2 | **Global trends of microbial biomass in the ocean and seabed.** **a** | The microbial biomass in the water column and surface sediments at station BIOTRANS in the north-east Atlantic[12]. **b** | The depth distribution of prokaryotic cells in a 140-metre-deep sediment core from the continental shelf off Peru (Ocean Drilling Program, leg 201, site 1227). Total cells detected by acridine orange direct counts are represented by blue squares; total numbers of bacteria are represented as pink spheres and total numbers of archaea are represented as yellow triangles, as determined by the quantitative PCR of 16S ribosomal RNA (rRNA) genes in extracted DNA. A similar quantification of eukaryotic 18S rRNA genes is shown for comparison (represented by stars). **c** | The relationship between microbial biomass in the surface sediments (0–15 cm) and the depth of the seafloor[11]. **d** | The relationship between microbial biomass in the surface sediments (0–1 cm) and the particulate organic carbon flux at the seafloor[11].

Hydrothermal vent ecosystems

In the early days of deep-sea oceanography, microbial life was thought to be restricted to the thin surface layer of the planet, where the organic matter derived from photosynthesis is present and is available as an energy and nutrient source, and where temperatures and chemical conditions are conducive to the known living organisms. The discovery of hydrothermal vent ecosystems, 30 years ago, established a completely new concept of the energy sources that are available for life in the deep ocean[25–28]. At hydrothermal vents, chemolithoautotrophic microorganisms are the primary producers in some of the most productive ecosystems on earth, because they can use a huge range of chemical compounds that are derived from hydrothermalism (BOX 2). Diverse and dense accumulations of animals depend on the energy flow through these chemoautotrophs. The icons of vent life are the symbiotic animals — the giant tubeworms, numerous molluscs and shrimps — that are permanently associated with the bacterial endo- and ectosymbionts that transform the chemical energy from vent fluids into food for their hosts. Owing to the extreme gradients and diversity in physical and chemical factors, hydrothermal vents remain incredibly fascinating to microbiologists, and most studies on deep-water microbial communities are now focused on vent habitats. Recent reviews of the biology and geochemistry of hydrothermal vent systems illustrate the tight coupling between geosphere and biosphere processes, as well as the immense heterogeneity of these intriguing ecosystems[29–31].

At hydrothermal vents, a group of highly reduced inorganic and organic compounds — the so called dark-energy or geofuels — are produced abiotically by magmatic degassing and subsurface water–rock reactions at high pressures and temperatures of up to 1,000°C (BOX 2). When the hot, electron-donor-rich vent fluids meet cold, electron-acceptor-rich ocean waters, the chemical energy becomes available to microorganisms and animal–microorganism symbioses that can exploit the many types of energetically favourable redox couples (BOX 2). Furthermore, where hot and cold fluids mix, dissolved materials precipitate and form energy-rich solid surfaces that can also be exploited by microorganisms through extracellular transport mechanisms that are only partially understood[32].

Most of the initial research on hydrothermal microbial life concentrated on the microbial diversity that is associated with vent plumes at mid-oceanic ridges (reviewed in REF. 29). Mid-oceanic ridge vent fluids share several geochemical characteristics, such as high temperatures of 300–400°C, an acidic pH, high (but highly variable) sulphide, methane, hydrogen and metal concentrations and an absence of sulphate[30,33]. Owing to the different tectonic and petrologic settings

of the Pacific, Atlantic and Indian mid-oceanic ridges, the geochemistry of vent fluids and precipitates varies between different vent systems, but the effects of the many different geological, physical and chemical factors on vent-fluid composition are not well understood. Vent fluids of fast-spreading ridges, such as the East Pacific Rise, are dominated by sulphide as the main electron donor for respiration, whereas vent fluids of the slow-spreading ridges, such as the Mid-Atlantic Ridge, provide most chemical energy as hydrogen and methane[34]. Hydrogen-driven vent habitats have now been found in a wide range of submarine geotectonic settings, such as ridge flanks, forearcs, backarcs, intraplate volcanoes, submarine volcanoes and active seamounts[29,35].

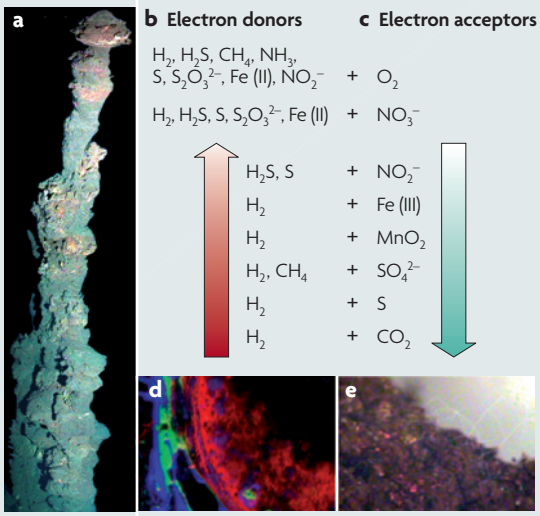
The discovery of the off-axis vent field named Lost City [36] caused particular excitement. Here, exposed mantle rock (peridotite) reacts with circulating seawater to produce hydrogen, and probably methane, at low temperatures. The relationship between abiotic and microbial methane production in serpentinization areas (serpentinization is the hydration and metamorphic transformation of ultramafic rock from the Earth's mantle into serpentine materials) remains a hot topic in hydrothermal vent research, particularly because these systems might be more widespread than previously thought. Other types of extreme vents are found at the iron-chemistry-dominated Loihi seamounts[37,38] and the carbon-dioxide vents of the Okinawa trough[39]. It will be a challenging task to investigate how distinct patterns in vent geochemistry influence microbial community composition and function, particularly because the within-vent heterogeneity might even exceed that between vent systems. On spatial scales of centimetres, and on temporal scales of minutes, the availability of certain redox couples can change by three orders of magnitude, and questions about the mutual controls between geochemistry, and microbial activity and diversity might only be solved by using appropriately high resolution and *in situ* sampling techniques.

Furthermore, the discovery of microbial corrosion structures and mineral alteration in ocean basalts, submarine lava, hydrothermal precipitates and vented rocks has shifted the focus from vent plumes to the huge undiscovered microbial realm in the seabed[40–44]. Challenging questions remain, such as what is the total seabed volume that is dominated by hydrothermal primary production and what is the total carbon-dioxide-fixation potential of the hydrothermal seabed, particularly because many vent habitats and novel autotrophic pathways remain unexplored.

Diversity at hot vents. Hydrothermal vents have been the environment of choice in the search for extremophiles, because of their combination of high temperatures,

Box 2 | **The microbial menu at hydrothermal vents**

Some bacteria and archaea have the ability to produce biomass by using chemical energy to fix carbon dioxide, through a process called chemosynthesis. However, most chemosynthetic life is not independent of photosynthesis: it depends on access to oxygen and nitrate as electron acceptors, and these are ultimately derived from photosynthesis. Chemoautotrophic microorganisms gain energy by oxidizing hydrogen, methane, hydrogen sulphide, ammonia, iron (II) and manganese (II), all of which occur in vent fluids. Depending on how they mix with seawater, CO_2, SO_4^{2-}, S, Fe (III), NO_3^- or O_2 can be available as oxidants.



In the figure, energy sources that are available at hydrothermal vents are shown. An extinct chimney, approximately 10 metres high, from the East Pacific Rise (EPR) 9°N is shown in panel **a**. Panels **b** and **c** show the redox couples of electron donors (in vent fluids) and electron acceptors (in bottom water). Panel **d** shows a tricolour display of the X-ray fluorescence map collected from the weathered-exterior (seawater-exposed) surface of the extinct hydrothermal chimney. Iron is coloured red, sulphur is coloured green and silicon is coloured blue. The light-green areas of the tricolour map are sulphide minerals. The red areas of the map are an iron-rich and sulphur-depleted weathering rind composed of iron oxides interspersed with silicon- and sulphur-rich precipitates. In panel **e**, a light-microscope image shows the weathered exterior of a sulphide structure colonized by filamentous bacteria from EPR 9°N (~10 x 10 mm).

Consortia of microorganisms control the rates of redox reactions, and modify their environment by producing biofilms and mats or establishing symbioses with animals. This enables them to access energy more easily and reliably, as they avoid being washed away by the currents. The different microbial metabolisms that occur at vents, the distributions of bacteria and archaea in relation to physico–chemical parameters such as temperature, pressure, pH, redox level and the presence or absence of other chemicals, is not yet well understood. The images in **a**, **d** and **e** are courtesy of K. J. Edwards, University of Southern California, California, USA and W. Bach, MARUM Research Centre Ocean Margin, Bremen, Germany.

Primary producer
An organism that is the original source of organic material in an ecosystem — plants, algae or chemosynthetic microorganisms.

Chemosynthesis
The biological conversion of 1 carbon molecule (usually carbon dioxide or methane) and nutrients into organic matter using the oxidation of inorganic molecules (for example, hydrogen gas or hydrogen sulphide) as a source of energy, rather than sunlight.

hydrostatic pressures, reducing power, toxic chemistry, extreme pH and enormous fluctuations of environmental conditions[29]. The heat-loving vent-archaea *Pyrolobus fumarii* and strain 121 hold the global record for growth at high temperatures (113 and 121°C, respectively)[45,46]. This temperature range is currently thought to represent the upper limit for life. A heterotrophic member of the ubiquitous, abundant and apparently endemic deep-sea hydrothermal vent euryarchaeota group DHVE2 was recently cultivated. It shows perfect adaptation to life at hydrothermal vents, with optimum growth at a low pH and high temperature, and the use of sulphur and iron as electron acceptors[47].

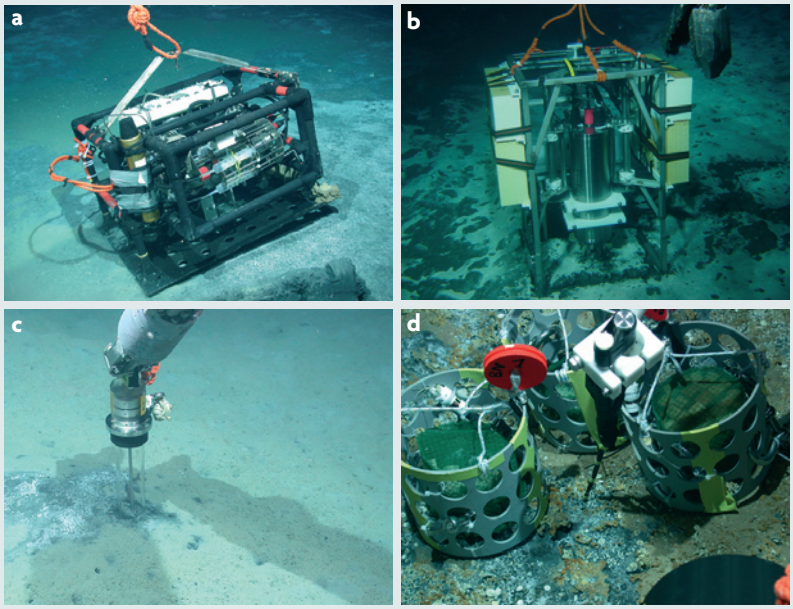
In addition to cultivation efforts, molecular biological methods, such as nucleic acid-based phylogenetic identification and genomic analyses of functional genes, have helped to advance research on microbial life at hydrothermal vents[29,48,49]. Archaea generally comprise a larger fraction of microbial communities at vents compared with ocean sediments. The deep-sea hydrothermal vent Euryarchaea group is abundant at vents, as well as the often cultured Thermococcales and Methanococcales[29]. Of the bacterial clades, the most widespread and abundant seem to be the epsilonproteobacteria[50], the chemolithoautotrophic Aquificales and diverse alpha- and gammaproteobacteria, which include the chemosynthetic endosymbionts of various tubeworms and bivalves[29]. The study of benthic microbial life at vents is a technological challenge, but new *in situ* colonization, drilling and sampling instruments might soon allow us to look deeper into these fascinating ecosystems, which are nourished by geofuel[48,51]. Understanding the source and fate of hydrogen in the vent environments, the microbial biosynthesis of hydrocarbons, the interaction of microorganisms with mineral surfaces and animal hosts and the dynamics of energy and carbon transport through different trophic levels remain hot topics in vent research.

Box 3 | **Technologies for biogeochemical *in situ* measurements**

A central question in vent and seep research is how the dark energy is used that is advected through the ocean crust and sediments. To determine the energy that is available to microbial communities from the oxidation of methane, sulphide, iron and other reduced compounds, fluxes of the redox couples in different microbial habitats must be quantified. This is not a trivial task, because fluid-flow-driven ecosystems, such as hot vents and cold seeps, are characterized by extremely steep and often highly variable chemical gradients over micrometre to centimetre ranges. Sample retrieval strongly alters the hydrostatic pressure and hence the chemical gradients and processes of the sample — particularly in gassy samples, which expand dramatically during retrieval to the ship. Therefore, there is an overriding requirement for *in situ* technologies that can be used to probe biogeochemical processes at the deep-ocean's hotspots.

Major achievements in understanding the variations in chemical gradients and fluxes at hydrothermal vents have included the use of video-guided *in situ* analytical instruments, such as sensors for temperature, pH and sulphide[117–119]. Biogeochemical processes in sedimentary systems can now be studied *in situ*, using independent underwater modules that are placed by submersibles and that autonomously measure and record data electronically. The figure shows examples of such modules, including a benthic chamber measurement of oxygen and methane fluxes at a mat patch (**a**), a microsensor profile of temperature, pH, redox, hydrogen sulphide and oxygen in a *Beggiatoa* spp. mat (**b**), the *in situ* sulphate reduction measurement in a small (30 cm) bacterial mat (**c**) and a colonization experiment monitoring microbial sulphide production (**d**).

Further technological challenges include the *in situ* quantification of gases, defined subsampling of hard rock, routine recovery of samples under *in situ* temperature and pressure for further analyses and experimentation, as well as the *in situ* fixation of samples for the analysis of sensitive molecules such as the various nucleic-acid products of microbial metabolisms[104]. The retrieval and maintenance of high-quality samples for geochemical, molecular and microbiological analyses depends on the availability of cold rooms, pressure vessels, anaerobic glove boxes and, especially, the expertise of the sampling party on board, who need to rapidly sub-sample, store, fix and freeze seafloor samples to preserve them. The images in **a–c** are courtesy of the MARUM Research Centre Ocean Margin, Bremen, Germany and the Max Planck Institute for Marine Microbiology, Bremen, Germany. The image in **d** is courtesy of the MARUM Research Centre Ocean Margin, Bremen, Germany and IFREMER (French Research Institute for Exploitation of the Sea), Brest, France.



Cold-seep ecosystems

A few years after the discovery of spectacular hydrothermal vent communities, a second type of chemosynthetic oasis, the cold seep, was discovered during submersible dives to the deep Gulf of Mexico[52] and to subduction zones of the Pacific[53,54]. Technological advances in high-resolution mapping and optical imaging, and *in situ* chemical analyses of the deep seafloor (BOX 3) have helped to reveal the vast diversity of hydrocarbon-fuelled chemosynthetic ecosystems, such as pockmarks, gas chimneys, mud volcanoes, brine ponds, and oil and asphalt seeps. These chemosynthetic ecosystems are usually found on continental margins at depths of 200–3,500 m and are characterized by the venting of hydrocarbons at fluid-flow velocities of a few tens of centimetres to a few metres per year[55–57]. Recently, various *in situ* chemical sensors and incubation chambers have been developed, which have been used to quantify the rates of microbial hydrocarbon turnover in seep ecosystems (BOX 3).

Diversity at cold seeps. A range of microorganisms can oxidize hydrocarbon compounds by using oxygen or sulphate as the terminal electron acceptor[58,59]. As oxygen is quickly depleted below the seafloor, subsurface anaerobes such as methanotrophs, hydrocarbon degraders and sulphate-reducing bacteria are the key functional

groups at cold-seep ecosystems. Unfortunately, environmentally relevant representatives of these bacterial and archaeal clades have not been cultivated. Hydrocarbon turnover, resulting in high sulphide fluxes at cold seeps, is usually dominated by sulphate-reducing bacteria of the deltaproteobacteria[60–62]. Cold-seep sediments host a high proportion of archaea, mainly methanotrophic Euryarchaeota and uncultured Crenarchaeota of the Marine Benthic Group B (also known as DSAG[21]) and C[61,63]. The microorganism-mediated anaerobic oxidation of methane (AOM) with sulphate, according to the equation $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$, is the dominant process at cold-seep ecosystems and one of the major global sinks of methane[64,65].

The analyses of 16S rRNA gene sequences and stable isotope signatures of specific biomarkers identified several phylogenetic clusters of archaea that are related to Methanosarcinales as the dominant anaerobic methanotrophs[60,66,67]. In most habitats that are fuelled by AOM, archaea form consortia with sulphate-reducing bacteria of the Desulfosarcina (Desulfococcus) or Desulfobulbus groups[57,60,67,68]. These associations are commonly attributed to obligate syntrophy, in which the archaeal partner activates and metabolizes methane, so providing an intermediate that is scavenged by the sulphate-reducing partner[69]. Analyses of carbon isotopes in seep ecosystems have shown a tight link between methane, methanotrophic archaea and their sulphate-reducing partners, authigenic carbonate precipitates and higher trophic levels in the food web[64,70,71]. Hence, similar to hot vents, cold seeps support an enormous biomass of free-living and symbiotic microbial life that is nourished by the oxidation of methane, higher hydrocarbons and sulphide[72–77]. In fact, methane-fuelled microbial communities in anoxic sediments above gas hydrates and gas vents have the highest biomass that is known to occur in marine ecosystems, with up to 10^{12} cells per cm^3 (REFS 68,78).

Seafloor architecture and cold-seep organisms. In many seep ecosystems, particularly those that remain active for tens of thousands of years, hard grounds form owing to the extensive carbonate precipitation that is a by-product of microbial hydrocarbon oxidation[68,79]. The fluid-flow regimes at cold-seep systems vary on small spatial scales of metres to hundreds of metres, and are host to different types of communities[55]. High fluid-flow rates of more than 5 m per year are often associated with gas ebullition, mud displacement and disturbed seafloor surfaces[57,80]. Owing to the absence of electron acceptors in subsurface fluids, such high fluid-flow velocities can restrict the microbial turnover of methane and sulphide to a few millimetres below the seafloor[81]. Medium flow rates are often associated with diverse types of microbial mats, some of which can cover hundreds of metres of seafloor (FIG. 3). These mats typically consist of giant, vacuolated sulphur-oxidizing bacteria, such as *Beggiatoa* and *Thiomargarita* spp., which exploit the high AOM-derived sulphide fluxes at the seafloor[82,83]. Such bacteria can use internally stored nitrate to oxidize sulphur and fix carbon dioxide for growth, thus coupling the carbon, nitrogen and sulphur cycles in the seep sediments. Their diversity is much higher than was previously anticipated, and each population has distinct adaptations to enable the use of the steep gradients of sulphide, nitrate and oxygen that develop in the methane-rich sediments[84]. Chemosynthetic bivalves and tubeworms dominate low fluid-flow systems, and show special adaptations to exploit subsurface sulphide and hydrocarbon pockets.

Gas hydrates and methane seepage. The discovery of huge subsurface reservoirs of methane, in the form of gas hydrates, has fuelled the interest in the associated seep ecosystems and their role as barriers against this potential greenhouse gas. The pore space of the seabed within the gas-hydrate stability zone consists of 1–10% gas hydrate on average, resulting in a global gas-hydrate reservoir of 1,000–22,000 gigatonnes of carbon[85]. Gas hydrates form when gas saturates the pore water at hydrostatic pressures of >60 bar and ambient temperatures of <4°C. According to the current understanding of carbon-isotope signatures, most of the methane that is stored in hydrates was formed by the microbial degradation of organic carbon by uncharacterized methanogens. Determining the origin of the methane and making quantitative measurements of hydrocarbon transport, turnover and emission are major challenges in seep research. Recent investigations of the microbial control of methane seepage have shown that fluid-flow velocity and access to electron acceptors such as sulphate, nitrate or oxygen are the main factors that structure microbial communities[57,81]. Advances in deep-sea technology, particularly in seafloor observation and mapping, have led to the discovery of highly diverse seep-related landscapes and habitats that have not yet been investigated for microbial diversity and function. New types of seeps, such as the intriguing carbon dioxide seeps of the Okinawa Trough[39] and asphalt volcanoes of the deep Gulf of Mexico[77], are still poorly understood in terms of the energy flow, composition and distribution of the key microbial populations that are hosted in these fascinating geo–bio-systems.

The deep subsurface biosphere

The first recognition of metabolically active microorganisms in deeply buried sediments arose from studies of pore-water chemistry in sediment cores that were obtained by drilling down to 150 m below the seafloor[86]. Towards the end of the 1980s, microbiological studies of the subsurface biosphere were enabled by the use of systematic acridine orange direct counts of total cell numbers[87]. Data compiled since then have been documented in an extensive database that contains information on the number and biomass of bacteria and archaea that were present in cores retrieved from many parts of the world's ocean[88]. The mean cell numbers varied greatly, but generally decreased from $1–5 \times 10^9$ cells per cm^3 near the sediment surface to 10^6 cells per cm^3 at the 1,000 m subsurface (FIG. 2). Rigorous

Pockmark
A depression in the seafloor 1–100 m in diameter that is presumably caused by eruptions of subsurface gases.

Gas chimney
A vertically extending circular anomaly (or blank) in the 3D seismic record of the seabed that indicates pathways of gas leakage.

Brine pond
A submarine accumulation of dense, salty seawater that leaks from the subsurface and fills depressions in the seabed.

Oil and asphalt seep
A natural leak of hydrocarbon (oil, tar or asphalt) from the deep seabed to the seafloor.

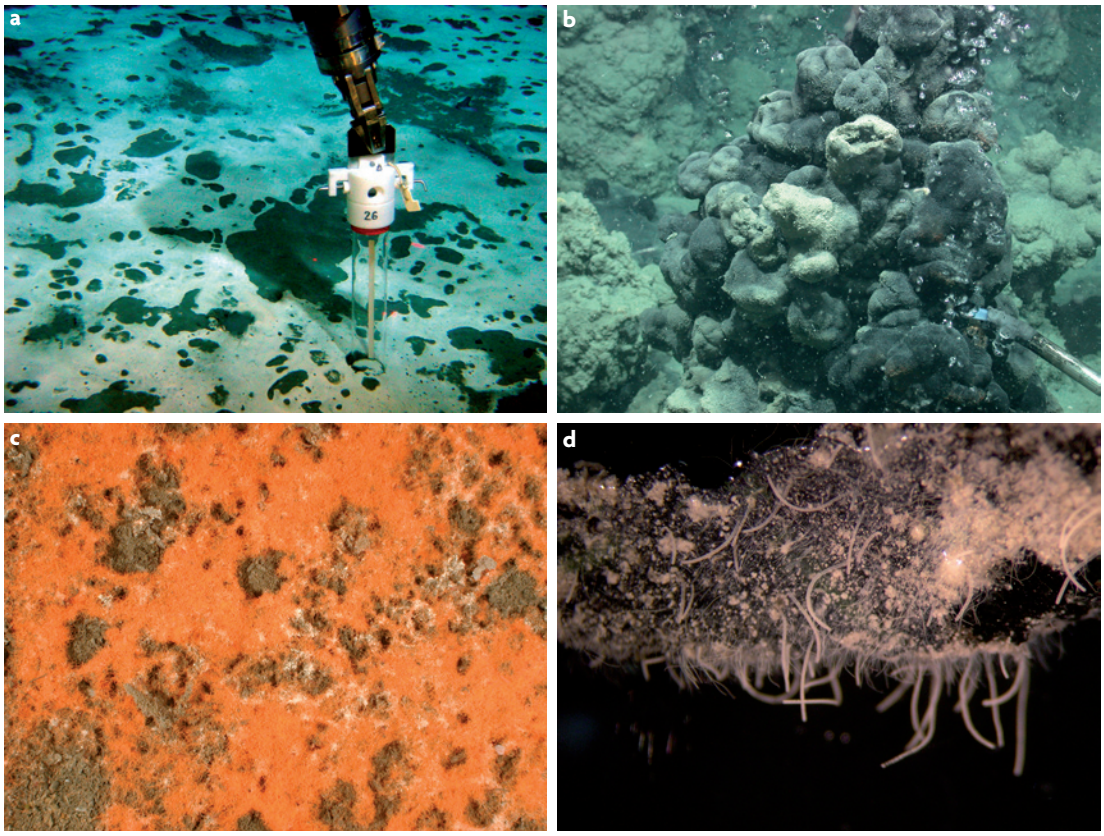


Figure 3 | **Microbial life at seep ecosystems. a** | A microbial mat at Haakon Mosby Mud Volcano. **b** | A methanotrophic microbial reef of the Black Sea. **c** | A microbial mat above oily sediments. **d** | A microbial mat on an asphalt flow. Image **a** is courtesy of IFREMER (French Research Institute for Exploitation of the Sea), Brest, France, image **b** is courtesy of the MARUM Research Centre Ocean Margin, Bremen, Germany, image **c** is courtesy of I. MacDonald, Texas A&M University, Corpus Christi, USA and image **d** is courtesy of F. Wenzhöfer, Max Planck Institute for Marine Microbiology, Bremen, Germany.

contamination tests that used the tracer perfluorocarbon in the drilling fluid and fluorescent microbeads at the drill head later confirmed that the cells counted were indigenous to the deep biosphere[89,90].

This discovery inspired Whitman and colleagues to make a global extrapolation of the cell numbers that are present in the entire seabed, ranging from 10 cm of the subsurface down to the ocean crust or at least to the expected thermal limit for microbial life, which is approximately 100°C[91]. Their astonishing conclusion was that the marine deep biosphere constituted the 'hidden majority' of all microbial cells, comprising between half and five-sixths of the Earth's total microbial biomass and between one-tenth and one-third of the Earth's total living biomass. Despite the high genetic diversity and physiological potential that this biosphere of bacteria and archaea might have, we still know relatively little about the biology and activity of its inhabitants. Research has continued rapidly since 2002, following the first cruise of the international Ocean Drilling Program (ODP), which was devoted to the exploration of the marine deep biosphere[92,93].

Bacterial and archaeal cells have been detected in nearly all marine sediments studied, ranging in age from recently sampled sediments to 37 million-year old deeply buried deposits of late Eocene age[94]. Owing to the geothermal gradient below the seafloor of 30–50°C per km, temperature sets an ultimate — and perhaps the only — limit to life at a depth of 2–4 km below the seafloor. Thus, sediments that have been buried to even greater depths, and are later geologically uplifted, seem to have been heat sterilized and no longer harbour living microorganisms[95]. This phenomenon is geochemically interesting, because it prevents the slow biodegradation of petroleum that takes place over geological timescales in oil reservoirs where temperatures are below 80°C. The gradual heating of sediments along with increasing depth of burial has another interesting effect — the activation of the recalcitrant buried organic matter and release of acetate[96].

Cultivation of deep biosphere microorganisms. Most deep-biosphere microorganisms detected so far have been extremely resistant to cultivation, which might be expected considering their incredibly slow *in situ* growth rates. The number of pure cultures that are available probably does not exceed a few hundred. Many different cultivation methods and media have been developed to try to mimic the environmental

conditions of the deep subsurface, such as using low substrate concentrations, supplying sterile sediment extracts, adding particles, maintaining low temperatures and monitoring cryptic growth using sensitive radiotracer techniques. The cultivation of microorganisms under high pressure has been achieved in only a few cases. The barophilic sulphate-reducing bacterium, *Desulfovibrio profundus*, was retrieved from samples that were taken from between 80 and 500 m sediment depths in the Japan Sea. This bacterium is unique because it grows over a broad mesophilic–thermophilic temperature range of 15–65°C[97]. A methanogenic archaeon, *Methanoculleus submarinus,* was isolated by Mikucki and colleagues[98] from samples that were obtained at 250 m below the subsurface in the Nankai Trough off Japan. More than 100 isolates belonging to at least 6 distinct lineages were isolated from a depth range of 1–400 m in subsurface sediments of the eastern tropical Pacific Ocean and the continental shelf off Peru[94]. Although many isolates are related to known marine organisms, some have a 16S rRNA gene sequence that differs by up to 14% from their nearest relative. Some cultivated lineages seem to have a cosmopolitan distribution in subsurface sediments, whereas others are consistently found only in low-organic matter deep-sea sediments or in high-organic matter sediments that are present at the continental margin. The most commonly cultured taxa are Firmicutes, which are closely related to the spore-forming *Bacillus firmus*, and alphaproteobacteria, which are closely related to *Rhizobium radiobacter*[94]. Rhizobia have also been isolated from organic rich sapropel layers in the Mediterranean seabed and seem to be abundant in the subsurface environment[99].

The predominant physiologies of the microorganisms that are present in deep sediments are expected to reflect the large-scale gradients of subsurface geochemistry, for example, gradients of sulphate or methane. However, from sequencing the 16S rRNA genes present in DNA that was extracted from ODP cores from different marine settings it was found that surprisingly few sequences could be related to known sulphate reducers or methanogens[100]. One of the few notable exceptions was a *Methanocaldococcus*-related phylotype that was detected below 200 m in the Peru Trench[22]. Members of the Methanobacteriales were also identified on the nearby continental shelf[101].

Diversity of deep biosphere life. Novel and uncultured lineages of bacterial and archaeal 16S rRNA genes dominate in clone libraries from the marine deep biosphere. The classical sulphate-reducing bacteria or methanogenic and methane-oxidizing archaea do not seem to constitute a significant fraction of the subsurface populations. There might be several reasons for this: first, they might occur in low population densities that are still sufficient to generate the sulphate and methane gradients observed; second, the processes might be carried out by novel phylogenetic lineages with divergent gene sequences; or third, difficulties in the quantitative extraction of DNA from deep sediments, which contain relatively low microbial biomass, might cause bias in the amplified genes[23,100].

Although no eukaryotes have been identified in deep-sediment samples, fragments of 18S rRNA genes from eukaryotic DNA have been extracted and sequenced[102] (FIG. 2). These might be derived from fossilized DNA, which could persist for thousands or millions of years either in inactive cells or in an extracellular form after cell death[103]. The transition from live to dead cells might be gradual owing to the energy limitation in the deep biosphere, and activities could range from actively growing cells, to cells that metabolize but do not grow, to intact cells that no longer produce biomolecules and finally to dead cells. Although environmental genomics, or single-cell genomics, might prove useful as a tool to characterize deep subsurface microorganisms, additional methods are required that can specifically target metabolically active populations[104]. One such method could be the quantification of intact phospholipids (IPL), which are diagnostic biomarkers of specific groups of bacteria or archaea that rapidly degrade after cell death[105]. The rRNA is also labile and can indicate cell viability. Cells with intact ribosomes can be counted by fluorescence *in situ* hybridization (FISH), which can be enhanced by intracellular catalysed-reporter deposition (CARD) for low-activity sediment bacteria[106]. It was concluded from studies using CARD-FISH in subsurface sediment samples from the Peru Margin that a minimum of 10–30% of the total cells were active bacteria, whereas active archaea were more scarce[107]. Another study at a nearby site, which combined IPL extraction, FISH and rRNA analysis, reached a different conclusion — up to 98% of the cells were archaea[23]. This showed that the specific quantification of subsurface microorganisms is still in its infancy, as it is not clear whether bacteria or archaea constitute the predominant domain of life in the deep biosphere.

Slow pace of life in the deep biosphere. For those microbiologists who are used to cultivating bacteria overnight, it could be difficult to grasp the slow pace of microbial processes in the subsurface seabed. Remains of deposited plankton debris that was buried 10 million-years ago are still being degraded at exceedingly slow rates by microbial populations that number millions per cm^3 (REF. 94). Interestingly, the total cell densities decrease by approximately sevenfold for each tenfold increase in the depth or age of the sediment[88]. By contrast, the reactivity of organic matter generally decreases by approximately tenfold for each tenfold increase in age[19]. Thus, the availability of organic matter per bacterial cell also decreases and drops by 100 to 1,000-fold from the sediment surface down to the ocean crust.

Superimposed on this general trend, alternating palaeoceanographic conditions have produced zones in the seabed of high organic deposition and burial. Local or large-scale oceanic anoxic events have produced intermittent sediment layers of extremely high organic content and with enhanced bacterial

activity and population density. Notable examples are the ~100,000-year old sapropel layers in Mediterranean deep-sea sediments or the 100 million-year old Cretaceous black shales that are buried half a kilometre beneath the equatorial Atlantic seafloor[108,109].

Based on the extremely low energy flux that could be available to individual cells, theoretical mean generation times calculated for deeply buried microorganisms range from years to thousands of years[107,110]. The existence of such large, but practically non-growing, populations challenges our understanding of the minimum energy requirements for life and the stability of the polymers that make up their biochemical machinery. For example, how can cells prevent or tolerate the unavoidable DNA damage? Are mutation rates higher or does the extremely slow growth favour the exchange of genetic information between cells rather than mutation within cells as the predominant mode of evolution? Alternatively, does the low energy availability constrain the ability of cells to repair mutations, thereby leading to faster evolution but also faster extinction[104]?

Energy sources that are independent of surface processes and photosynthesis have been proposed to explain the conundrum of slow growth. Hydrogen is produced in the subsurface when anoxic seawater reacts with iron-bearing igneous rocks and is an optimal source of dark energy for lithotrophic organisms[111]. Although this source of energy is important in the fresh basalt along the mid-oceanic ridges, the rates of hydrogen formation in the old- and cold-ocean crust seem to be too low to contribute significant energy relative to the energy that is derived from buried organic material[94,110,112]. An alternative hydrogen source, that has received increasing attention in recent years, might arise from the interaction of natural radioactivity with water[113,114]. The radioisotopes ^{40}K, ^{232}Th and ^{238}U, which are common in marine sediments, generate hydrogen, hydrogen peroxide, hydroxide radicals and other highly reactive products by the radiolysis of water. Thus, this energy source could provide small amounts of oxygen for aerobic bacteria, and hydrogen as energy for either aerobes or anaerobes. However, compared with ancient organic matter, it is still only in the most energy depleted deep-sea sediments that radiolysis might be the main energy source.

Outlook

In the study of the carbon cycle and the cycling of other elements in the sea, microbial populations and their functions have often been categorized as a black box. The outdated view of a relatively uniform seafloor, structured only by a few physico–chemical parameters such as pressure, temperature, oxygen or energy availability, has been replaced by that of a large diversity of seafloor landscapes and geo–bio-systems, all of which host unique microbial communities. However, the enormous diversity of microbial catalytic capabilities has not been completely explored and expands continually as new organisms are discovered. Only a tiny fraction of benthic microorganisms have been identified, and even fewer have been obtained in pure culture. We do not know the identity of most of the microorganisms that are responsible for the cycling of carbon, nitrogen, sulphur, manganese, iron and silicon in the seafloor. It is possible that a small number of important microorganisms dominate element cycling, in the same way that just a few algal species are responsible for a large fraction of primary production and POM export in the pelagic realm. Geochemical data has revealed novel microbial processes, such as ethanogenesis and propanogenesis, that were previously assumed to be thermochemical reactions of fossilized organic material[115]. Deep-biosphere research in particular has profoundly altered our perspective on the limits of living organisms and their need for energy. How is it possible to maintain complex functions in microbial cells at an energy flux that barely allows cell growth over tens to thousands of years? Price and Sowers[116] found that the metabolic rates per cell that corresponded to growth, maintenance and survival were on a scale of 10^6:10^3:1, respectively. Thus, there is a millionfold difference between metabolic rates in laboratory cultures and those in some of the largest ecosystems on Earth — the terrestrial and marine subsurface, the permafrost and the ice. It is a challenge for the future to understand microbial life under such extremely energy-depleted conditions.

New approaches to the study of seafloor ecosystems have been facilitated by recent advances in ocean technology, particularly by the use of a range of underwater vehicles such as manned, unmanned and autonomous submersibles and new drilling tools. Owing to the threat of the rapid destruction of habitats and extinction of species in connection to global change and anthropogenic impacts on the ocean, large programmes such as the Census of Marine Life and the EU project HERMES (Hot spot ecosystems on margins of European Seas) (see Further information) have been initiated to study the diversity of ocean life, including microbial biodiversity (for example, the International Census of Marine Microbes; see Further information). New molecular methods now allow the investigation of environmental microbial biodiversity using high-throughput genomic methods, but so far most of these studies have been restricted to ocean waters. Only four ocean-floor metagenomes are in progress, all of which target chemosynthetic ecosystems such as seeps, vents and whale falls (for example, the Genomes OnLine Database v 2.0; see Further information).

Comparable efforts have not yet been made for the wider seafloor realm, and even the simplest questions about patterns in microbial biodiversity and biogeography cannot be answered, although the necessary methods and technologies are available. Taking into account the low turnover of deep-sea benthic microbial communities, which results from limited energy supplies and large-standing stocks of carbon, the environmental factors that drive microbial diversity at the ocean floor and the spatial and temporal scales on which they operate remain fascinating questions that are highly pertinent to microbial evolution.

1. Zobell, C. E. & Morita, R. Y. in *Galathea Report* Vol. 1 139–154 (Danish Science, Copenhagen, 1959).
2. Corliss, J. B. *et al.* Submarine thermal springs on the Galapagos Rift. *Science* **203**, 1073–1083 (1979).
3. Wenzhöfer, F. & Glud, R. N. Benthic carbon mineralization in the Atlantic: a synthesis based on *in situ* data from the last decade. *Deep-Sea Res.I* **49**, 1255–1279 (2002).
4. Froelich, P. N. *et al.* Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochim. Cosmochim. Acta* **43**, 1075–1090 (1979).
5. Jahnke, R. A. & Jackson, G. A. in *Deep-Sea Food Chains and the Global Carbon Cycle* (eds Rowe, G. T. & Pariente, V.) 295–308 (Kluwer Academic, Dordrecht, 1992).
6. Mayer, L. in *Organic Geochemistry: Principles and Applications* (eds Engel, M. H. & Macko, S.) 171–184 (Plenum, New York, 1993).
7. Hedges, J. I. & Keil, R. G. Sedimentary organic matter preservation: an assessment and speculative synthesis. *Mar. Chem.* **49**, 81–115 (1995).
8. Deming, J. W. & Baross J. A. in *Organic Geochemistry* (eds Engel, M. H. & Macko, S. A.) 119–144 (Plenum, New York, 1993).
9. Smith, C. R. *et al.* Latitudinal variations in benthic processes in the abyssal equatorial Pacific: control by biogenic particle flux. *Deep-Sea Res. II* **44**, 2295–2317 (1997).
10. Boetius, A. & Damm, E. Benthic oxygen uptake, hydrolytic potentials and microbial biomass at the Arctic continental slope. *Deep-Sea Res. I* **45**, 239–275 (1998).
11. Deming, J. W. & Yager, P. L. in *Deep-Sea Food Chains and the Global Carbon Cycle* (eds Rowe, G. T. & Pariente, V.) 11–28 (Kluwer Academic, Dordrecht, 1992).
Together with reference 12, this reference was important for the understanding of global patterns in microbial biomass and activity in deep-sea sediments.
12. Lochte, K. in *Deep-Sea Food Chains and the Global Carbon Cycle* (eds Rowe, G. T. & Pariente, V.) 1–10 (Kluwer Academic, Dordrecht, 1992).
13. Lochte, K. & Turley, C. M. Bacteria and cyanobacteria associated with phytodetritus in the deep-sea. *Nature* **333**, 67–69 (1988).
14. Turley, C. M. & Lochte, K. Microbial response to the input of fresh detritus to the deep-sea bed. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **89**, 3–23 (1990).
This paper was one of the first to provide a quantification of the microbial turnover of phytodetritus at the seafloor and showed that deep-sea microorganisms can be as active as other microorganisms despite cold temperatures and high pressures.
15. Moodley, L. *et al.* Bacteria and Foraminifera: key players in a short-term deep-sea benthic response to phytodetritus. *Mar. Ecol. Prog. Ser* **236**, 23–29 (2002).
16. Witte U. *et al. In situ* experimental evidence of the fate of a phytodetritus pulse at the abyssal seafloor. *Nature* **424**, 763–766 (2003).
17. Vetter, Y. A. & Deming, J. Extracellular enzyme activity in the Arctic Northeast Water polynya. *Mar. Ecol. Prog. Ser* **114**, 23–34 (1994).
18. Boetius, A. & Lochte, K. Regulation of microbial enzymatic degradation of OM in deep-sea sediments. *Mar. Ecol. Prog. Ser* **104**, 299–307 (1994).
19. Burdige, D. J. Preservation of organic matter in marine sediments: controls, mechanisms, and an imbalance in sediment organic carbon budgets. *Chem. Rev.* **107**, 467–485 (2007).
20. DeLong, E. F., Franks, D. G. & Yayanos A. A. Evolutionary relationships of cultivated psychrophilic and barophilic deep-sea bacteria. *Appl. Environ. Microbiol.* **63**, 2105–2108 (1997).
21. Vetriani, C., Jannasch, H. W., MacGregor, B. J., Stahl, D. A. & Reysenbach, A. L. Population structure and phylogenetic characterization of marine benthic archaea in deep-sea sediments. *Appl. Environ. Microbiol.* **65**, 4375–4384 (1999).
22. Inagaki, F. *et al.* Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl Acad. Sci. USA* **103**, 2815–2820 (2006).
23. Biddle, J. F. *et al.* Heterotrophic archaea dominate sedimentary subsurface ecosystems off Peru. *Proc. Natl Acad. Sci. USA* **103**, 3846–3851 (2006).
24. Middelboe, M., Glud, R. N., Wenzhöfer, F., Oguri, K., Kitazato, H. Spatial distribution and activity of viruses in the deep-sea sediments of Sagami Bay, Japan. *Deep-Sea Res. I* **53**, 1–13 (2006).
25. Lonsdale, P. Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at oceanic spreading centers. *Deep-Sea Res.* **24**, 857–863 (1977).
26. Jones, M. L. Hydrothermal vents of the eastern Pacific: an overview. *Bull. Biol. Soc. Wash.* **6**, 545 (1985).
27. Cavanaugh, C. M. Symbioses of chemoautotrophic bacteria and marine invertebrates from hydrothermal vents and reducing sediments. *Bull. Biol. Soc. Wash.* **6**, 373–388 (1985).
28. Nelson, D. C., Wirsen, C. O. & Jannasch, H. W. Characterization of large autotrophic Beggiatoa abundant at hydrothermal vents of the Guaymas Basin. *Appl. Environ. Microbiol.* **55**, 2909–2917 (1989).
29. Takai, K., Nakagawa, S., Reysenbach, A. L. & Hoek, J. in *Back Arc Spreading Systems: Geological, Biological, Chemical and Physical Interactions* (eds Christie, D. M. *et al.*) 185–214 (AGU Books, Washington DC, 2006).
An important contribution to the documentation of the occurrence of uncultivated microorganisms at different hydrothermal vent systems.
30. Tivey, M. Generation of seafloor hydrothermal vent fluids and associated mineral deposits. *Oceanography* **20**, 50–65 (2007).
31. Fisher, C. R., Takai, K. & Le Bris, N. Hydrothermal vent ecosystems. *Oceanography* **20**, 14–23 (2007).
32. Edwards, K. J., Bach, W. & McCollom, T. M. Geomicrobiology in oceanography: microbe–mineral interactions at and below the seafloor. *Trends Microbiol.* **13**, 449–456 (2006).
33. Von Damm, K. L. *et al.* Chemistry of submarine hydrothermal solutions at 21°N, East Pacific Rise. *Geochim. Cosmochim. Acta* **49**, 2197–2220 (1985).
34. Charlou, J. L. *et al.* Geochemistry of high H_2 and CH_4 vent fluids issuing from ultramafic rocks at the Rainbow hydrothermal field (36°14'N, MAR). *Chem. Geol.* **191**, 345– 359 (2002).
35. Nealson, K. H., Inagaki, F. & Takai, K. Hydrogen-driven subsurface lithoautotrophic microbial ecosystems (SLiMEs): do they exist and why should we care? *Trends Microbiol.* **13**, 405–410 (2005).
36. Kelley, D. S. *et al.* A serpentinite-hosted ecosystem: the lost city hydrothermal field. *Science* **307**, 1428–1434 (2005).
37. Moyer, C. L., Tiedge, J. M., Dobbs, F. C. & Karl, D. M. Diversity of deep-sea hydrothermal vent Archaea from Loihi Seamount, Hawaii. *Deep-Sea Res. II* **45**, 303–317 (1998).
38. Emerson, D. & Moyer. C. L. Neutrophilic Fe-oxidizing bacteria are abundant at the Loihi Seamount hydrothermal vents and play a major role in Fe oxide deposition. *Appl. Environ. Microbiol.* **68**, 3085–3093 (2002).
39. Inagaki, F. *et al.* Microbial community in a sediment-hosted CO_2 lake of the southern Okinawa Trough hydrothermal system. *Proc. Natl Acad. Sci. USA* **103**, 14164–14169 (2006).
40. Fisk, M. R., Giovannoni, S. J. & Thorseth, I. H. Alteration of oceanic volcanic glass: textural evidence of microbial activity. *Science* **281**, 978–980 (1998).
41. Furnes, H. & Staudigel, H. Biological mediation in ocean crust alteration: how deep is the deep biosphere? *Earth Planet. Sci. Lett.* **166**, 97–103 (1999).
42. Thorseth, I. H. *et al.* Diversity of life in ocean floor basalt. *Earth. Planet. Sci. Lett.* **194**, 31–37 (2001).
43. Bach, W. & Edwards, K. J. Iron and sulfide oxidation within the basaltic ocean crust: implications for chemolithoautotrophic microbial biomass production. *Geochim. Cosmochim. Acta* **67**, 3871–3887 (2003).
44. Shock, E. L. & Holland M. E. Geochemical energy sources that support the subsurface biosphere. *Geophysical Monograph* **144**, 469–525 (2004).
45. Blöchl, E. *et al. Pyrolobus fumarii* gen. and sp. nov., represents a novel group of archaea extending the upper temperature limit for life to 113°C. *Extremophiles* **1**, 14–21 (1997).
46. Kashefi, K. & Lovely, D. R. Extending the upper temperature limit for life. *Science* **301**, 934 (2003).
47. Reysenbach, A. L. *et al.* A ubiquitous thermoacidophilic archaeon from deep-sea hydrothermal vents. *Nature* **442**, 444–447 (2006).
A perfect example of the successful cultivation of vent-endemic environmentally relevant archaea based on their distribution, the metagenome and the geochemistry of their habitat.
48. Reysenbach, A. L., Longnecker, K. & Kirshtein, J. Novel bacterial and archaeal lineages from an *in situ* growth chamber deployed at a Mid-Atlantic ridge hydrothermal vent. *Appl. Environ. Microbiol.* **66**, 3798–3806 (2000).
49. Reysenbach, A. L. & Shock, E. L. Merging genomes with geochemistry in hydrothermal ecosystems. *Science* **296**, 1077–1082 (2002).
50. Campbell, B., Engel, A. S., Porter, M. L. & Takai, K. The versatile ε-proteobacteria: key players in sulphidic habitats. *Nature Rev. Microbiol.* **4**, 458–468 (2006).
51. Bach, W. *et al.* Energy in the dark: fuel for life in the deep ocean and beyond. *Eos (Washington DC)* **87**, 73–78 (2006).
52. Kennicutt, M. C. *et al.* Vent-type taxa in a hydrocarbon seep region on the Louisiana slope. *Nature* **317**, 351–353 (1985).
53. Suess, E. *et al.* Biological communities at vent sites along the subduction zone off Oregon. *Bull. Biol. Soc. Wash.* **6**, 475–484 (1985).
54. Kulm, L. D. *et al.* Oregon subduction zone: venting, fauna, and carbonates. *Science* **231**, 561–566 (1986).
55. Tryon, M. D. *et al.* Measurements of transience and downward fluid flow near episodic methane gas vents, Hydrate Ridge, Cascadia. *Geology* **27**, 1075–1078 (1999).
56. Boetius, A. & Suess, E. Hydrate Ridge: a natural laboratory for the study of microbial life fueled by methane from near-surface gas hydrates. *Chem. Geol.* **205**, 291–310 (2004).
57. Niemann, H. *et al.* Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. *Nature* **443**, 854–858 (2006).
This paper combines geochemistry and molecular ecology *in situ* to provide a quantitative understanding of the environmental factors that control the microbial methane filter.
58. Orphan, V. J., Haffenbradl, D., Taylor, L. T. & Delong, E. F. Culture-dependent and culture-independent characterization of microbial assemblages associated with high temperature petroleum reservoirs. *Appl. Environ. Microbiol.* **66**, 700–711 (2000).
59. Widdel, F., Musat, F., Knittel, K. & Galushko, A. in *Sulphate-Reducing Bacteria: Environmental and Engineered Systems.* (eds Barton, L. L. & Hamilton, W. A.) 265–304 (Cambridge Univ. Press, Cambridge, 2007).
60. Orphan, V. J., House, C. H., Hinrichs, K. U., McKeegan, K. D. & Delong, E. F. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *Proc. Natl Acad. Sci. USA* **99**, 7663–7668 (2002).
61. Teske, A. *et al.* Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. *Appl. Environ. Microbiol.* **68**, 1994–2007 (2002).
62. Knittel, K. *et al.* Activity, distribution, and diversity of sulfate reducers and other bacteria in sediments above gas hydrate (Cascadia Margin, Oregon). *Geomicrobiol. J.* **20**, 269–294 (2003).
63. Knittel, K., Lösekann, T., Boetius, A., Kort, R. & Amann, R. Diversity and distribution of methanotrophic archaea at cold seeps. *Appl. Environ. Microbiol.* **71**, 467–479 (2005).
64. Hinrichs, K. U. & Boetius, A. in *Ocean Margin Systems* (eds Wefer, G. *et al.*) 457–477 (Springer-Verlag, Berlin, 2002).
65. Reeburgh, W. Oceanic methane biogeochemistry. *Chem. Oceanogr.* **107**, 486–513 (2007).
66. Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. G. & DeLong, E. F. Methane-consuming archaebacteria in marine sediments. *Nature* **398**, 802–805 (1999).
A classical example of how cultivation-independent molecular methods such as lipid biomarkers and 16S rDNA analyses can help to identify unknown microorganisms and their metabolisms. Also, this paper was the first to provide clues to the identity of anaerobic methanotrophs at seeps.

67. Boetius, A. *et al.* A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* **407**, 623–626 (2000).
68. Michaelis, W. *et al.* Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science* **297**, 1013–1015 (2002).
69. Valentine, D. L. & Reeburgh, W. S. New perspectives on anaerobic methane oxidation. *Environ. Microbiol.* **2**, 477–484 (2000).
70. Ritger, S., Carson, B. & Suess, E. Methane-derived authigenic carbonates formed by subduction-induced pore-water expulsion along the Oregon/Washington margin. *Geol. Soc. Am Bull.* **98**, 147–156 (1987).
71. Levin, L. Ecology of cold seep ecosystems: interactions of fauna with flow, chemistry and microbes. *Oceanogr. Mar. Biol. Annu. Rev.* **43**, 1–46 (2005).
72. MacDonald, I. R. *et al.* Gulf of Mexico chemosynthetic communities: II. Spatial distribution of seep organisms and hydrocarbons at Bush Hill. *Mar. Biol.* **101**, 235–247 (1989).
73. Fisher, C. R. Chemoautotrophic and methanotrophic symbioses in marine invertebrates. *Rev. Aquat. Sci.* **2**, 399–436 (1990).
74. Sibuet, M. & Olu, K. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. *Deep-Sea Res. II* **45**, 517–567 (1998).
75. Sahling, H., Rickert, D., Lee, R. W., Linke, P. & Suess, E. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Mar. Ecol. Prog. Ser* **231**, 121–138 (2002).
76. Joye, S. B. *et al.* The anaerobic oxidation of methane and sulfate reduction in sediments from Gulf of Mexico cold seeps. *Chem. Geol.* **205**, 219–238 (2004).
77. MacDonald, I. R. *et al.* Asphalt volcanism and chemosynthetic life in the Campeche Knolls, Gulf of Mexico. *Science* **304**, 999–102 (2004).
78. Treude, T. *et al.* Consumption of methane and CO_2 by methanotrophic microbial mats from gas seeps of the anoxic Black Sea. *Appl. Environ. Microbiol.* **73**, 2271–2283 (2007).
79. Hovland, M. & Judd, A. G. *Seabed Pockmarks and Seepages* (Graham and Trotman, London, 1988).
80. Dupré, S. *et al.* Seafloor geological studies above active gas chimneys off Egypt (Central Nile Deep Sea Fan). *Deep-Sea Res. I* **54**, 1146–1172 (2007).
81. de Beer, D., Sauter, E., Niemann, H., Witte, U. & Boetius, A. *In situ* fluxes and zonation of microbial activity in surface sediments of the Håkon Mosby Mud Volcano. *Limnol. Oceanogr.* **51**, 1315–1331 (2006).
82. Treude, T., Boetius, A., Knittel, K., Wallmann, K. & Jørgensen, B. B. Anaerobic oxidation of methane above gas hydrates at Hydrate Ridge, NE Pacific. *Mar. Ecol. Prog. Ser* **264**, 1–14 (2003).
83. Kalanetra, K. M., Joye, S. B., Sunseri, N. R. & Nelson, D. C. Novel vacuolate sulfur bacteria from the Gulf of Mexico reproduce by reductive division in three dimensions. *Environ. Microbiol.* **7**, 1451–1460 (2005).
84. Preisler, A. *et al.* Biological and chemical sulfide oxidation in a *Beggiatoa* inhabited sediment. *ISME J.* **1**, 341–353 (2007).
85. Dickens, G. Rethinking the global carbon cycle with a large, dynamic and microbially mediated gas hydrate capacitor. *Earth Planet. Sci. Lett.* **213**, 169–183 (2003).
86. Oremland, R. S., Culbertson, C. & Simoneit, B. R. T. in *Init. Repts. DSDP* (eds Curray, J. R. *et al.*) 759–762 (United States Government Printing Office, Washington, 1982).
87. Cragg, B. A. *et al.* in *Proc. Ocean Drilling Program Sci.* (eds Suess, E. & von Huene, R.) Vol. 112, 607–619 (A&M University, Texas, 1990).
88. Parkes, R. J., Cragg, B. A. & Wellsbury, P. Recent studies on bacterial populations and processes in marine sediments: a review. *Hydrogeol. J.* **8**, 11–28 (2000).
89. Smith, D. C., Spivack, A. J., Fisk, M. R., Haveman, S. A. & Staudigel, H. Tracer-based estimates of drilling-induced microbial contamination of deep sea crust. *Geomicrobiol. J.* **17**, 207–219 (2000).
90. House, C. H., Cragg, B. A. & Teske, A. in *Proc. Ocean Drilling Program, Init. Repts.* (eds D'Hondt, S. L. *et al.*) Vol. 201, 1–19 (A&M University, Texas, 2003).
91. Whitman, W. B., Coleman, D. C. & Wiebe, W. J. Prokaryotes: the unseen majority. *Proc. Natl Acad. Sci. USA* **95**, 6578–6583 (1998).
A key paper on the global abundance and biomass of prokaryotic cells, which showed their predominance in the deep biosphere.
92. D' Hondt, S. L. *et. al.* Metabolic activity of subsurface life in deep-sea sediments. *Science* **295**, 2067–2070 (2002).
93. D' Hondt, S. L. *et al.* (eds) in *Proc. Ocean Drilling Program, Init. Repts.* Vol. 201, 1–86 (Texas A&M University, Texas, 2003).
94. D' Hondt, S. *et al.* Distributions of microbial activities in deep subseafloor sediments. *Science* **306**, 2216–2221 (2004).
An important overview of the data on microbial diversity and metabolic rates in a range of sediments from the continental shelf to the deep sea.
95. Wilhelms, A. *et al.* Biodegradation of oil in uplifted basins prevented by deep-burial sterilization. *Nature* **411**, 1034–1037 (2001).
96. Wellsbury, P. *et al.* Deep bacterial biosphere fuelled by increasing organic matter availability during burial and heating. *Nature* **388**, 573–576 (1997).
97. Bale, S. J. *et al. Desulfovibrio profundus* sp. nov., a novel barophilic sulphate-reducing bacteria from deep sediment layers in the Japan Sea. *Int. J. Syst. Bacteriol.* **47**, 515–521 (1997).
98. Mikucki, J. A., Liu, Y., Delwiche, M., Colwell, F. S. & Boone, D. R. Isolation of a methanogen from deep marine sediments that contain methane hydrates, and description of *Methanoculleus submarinus* sp. nov. *Appl. Environ. Microbiol.* **69**, 3311–3316 (2003).
99. Süss, J. *et al.* Widespread distribution and high abundance of *Rhizobium radiobacter* within Mediterranean subsurface sediments. *Environ. Microbiol.* **8**, 1753–1763 (2006).
100. Teske, A. Microbial communities of deep marine subsurface sediments: molecular and cultivation surveys. *Geomicrobiol. J.* **23**, 357–368 (2006).
101. Parkes, R. J. *et al.* Deep subsurface prokaryotes stimulated at interfaces over geologic time. *Nature* **436**, 390–394 (2005).
102. Schippers, A. & Neretin, L. N. Quantification of microbial communities in near-surface and deeply buried marine sediments on the Peru continental margin using real-time PCR. *Environ. Microbiol.* **8**, 1251–1260 (2006).
103. Coolen, M. J. L. & Overmann, J. Analysis of subfossil molecular remains of purple sulfur bacteria in a lake sediment. *Appl. Environ. Microbiol.* **64**, 4513–4521 (1998).
104. D'Hondt, S. *et al.* (eds) in *Workshop Report of the Integrated Ocean Drilling Program* (A&M University, Texas, in the press).
105. Sturt, H. F., Summons, R. E., Smith, K., Elvert, M. & Hinrichs, K. U. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry — new biomarkers for biogeochemistry and microbial ecology. *Rapid Commun. Mass Spectrom.* **18**, 617–628 (2004).
106. Pernthaler, A., Pernthaler, J. & Amann, R. Fluorescence *in situ* hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Appl. Environ. Microbiol.* **68**, 3094–3101 (2002).
107. Schippers, A. *et al.* Prokaryotic cells of the deep subsurface biosphere identified as living bacteria. *Nature* **433**, 861–864 (2005).
108. Coolen, M. J. L., Cypionka, H., Sass, A. M., Sass, H. & Overmann, J. Ongoing modification of Mediterranean Pleistocene sapropels mediated by prokaryotes. *Science* **296**, 2407–2410 (2002).
109. Arndt, S., Brumsack, H. J. & Wirtz, K. W. Cretaceous black shales as active bioreactors: a biogeochemical model for the deep biosphere encountered during ODP Leg 207 (Demerara Rise). *Geochim. Cosmochim. Acta* **70**, 408–425 (2006).
110. Jørgensen, B. B., D'Hondt, S. L. & Miller, D. J. in *Proc. Ocean Drilling Program, Init. Repts.* (eds Jørgensen, B. B., D'Hondt, S. L. & Miller, D. J.) Vol. 201, 1–45 (Texas A&M University, Texas, 2006).
111. Schulte, M., Blake, D., Hoehler, T. & McCollom, T. Serpentinization and its implications for life on the early Earth and Mars. *Astrobiology* **2**, 364–376 (2006).
112. Jørgensen, B. B. & D'Hondt, S. A starving majority deep beneath the seafloor. *Science* **314**, 932–934 (2006).
113. Lin, L. H., Slater, G. F., Lollar, B. S., Lacrampe-Couloume, G. & Onstott, T. C. The yield and isotopic composition of radiolytic H_2, a potential energy source for the deep subsurface biosphere. *Geochim. Cosmochim. Acta* **69**, 893–903 (2005).
114. Blair, C., D'Hondt, S., Spivack, A. J. & Kingsley, R. H. Radiolytic hydrogen and microbial respiration in a deep sea sediment column. *Astrobiology* **6**, 198 (2006).
115. Hinrichs, K. U. *et al.* Biological formation of ethane and propane in the deep marine subsurface. *Proc. Natl Acad. Sci. USA* **103**, 14684–14689 (2006).
116. Price, P. B. & Sowers, T. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proc. Natl Acad. Sci. USA* **101**, 4631–4636 (2004).
117. Johnson, K. S., Childress, J. J. & Beelher, C. L. Short time temperature variability in the Rose Garden hydrothermal vent field: an unstable deep-sea environment. *Deep-Sea Res. A* **35**, 1711–1721 (1988).
118. Luther, G. W. *et al.* Chemical speciation drives hydrothermal vent ecology. *Nature* **410**, 813–816 (2001).
119. Le Bris, N., Zbinden, M. & Gaill, F. Processes controlling the physico-chemical micro-environments associated with Pompeii worms. *Deep-Sea Res. I* **52**, 1071–1083 (2005).

Acknowledgments
Financial support for writing this review was provided to the laboratory of B.B.J. by the Max Planck Society and the Fonds der Chemischen Industrie, and to the laboratory of A.B. by the EU FP6 program HERMES (Hot spot ecosystem research on ocean margins of European Seas). We thank K. Edwards, W. Bach, N. Dubilier, J. Harder and F. Wenzhöfer for helpful comments and seafloor images, and A. Schippers for providing published data.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Bo Barker Jørgensen's homepage: http://www.mpi-bremen.de/en/Biogeochemistry_Group.html
Antje Boetius's homepage: http://www.mpi-bremen.de/en/Antje_Boetius.html
Census of Marine Life: www.coml.org
Genomes OnLine Database v 2.0: http://genomesonline.org/
HERMES: www.eu-hermes.net
International Census of Marine Microbes: http://icomm.mbl.edu/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF