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Microbial Ecology of the Dark Ocean above, at, and below the Seafloor†

Beth N. Orcutt,[1,2] Jason B. Sylvan,[2] Nina J. Knab,[2] and Katrina J. Edwards[2,3]*

Center for Geomicrobiology, Aarhus University, 8000 Aarhus, Denmark[1]; Marine Environmental Biology Section, Department of Biological Sciences, University of Southern California, Los Angeles, California 90089[2]; and Department of Earth Sciences, University of Southern California, Los Angeles, California 90089[3]

* Corresponding author. Mailing address: Department of Biological Sciences, University of Southern California, 3616 Trousdale Blvd., Los Angeles, CA 90089. Phone: (213) 821-4390. Fax: (213) 740-8123. E-mail: kje@usc.edu.

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INTRODUCTION

The majority of the Earth's habitable environments are physically located in environments that do not receive sunlight. Indeed, the largest potential habitats on Earth are located in the ocean, which covers approximately 70% of the Earth's surface (Fig. 1). The ocean's average depth is 4,000 m and reaches as deep as 11,000 m in the Marianas Trench. Depending on the quantity of particulate matter in the water, significant levels of sunlight—the driver of photosynthesis—penetrate only a few tens to hundreds of meters (300 m maximum) into the water. Thus, from a quantitative perspective, most of the ocean—roughly 1.27×10^{18} m^3—exists in the dark, with a minor volume (3.0×10^{16} m^3) actually penetrated by sunlight (Table 1). Considering that other habitats exist beneath the ocean water column, such as marine sediments, oceanic crust, and hydrothermal vents (Table 1), these dark ocean environments (Fig. 2 to 5) together comprise the largest collection of habitats by volume that life—in particular, microbial life—can occupy on Earth.

Our knowledge of microbial processes in the dark ocean has increased enormously in recent decades, owing in part to the exciting discoveries of hydrothermal vents, cold seeps, and whale falls at the bottom of the ocean in the late 1970s and 1980s (91, 249, 439, 504). Studies that try to decipher the activity of microorganisms in the dark ocean, where we cannot easily observe them, continually yield paradigm-shifting discoveries, fundamentally changing our understanding of the role of the dark ocean in the global Earth system and its biogeochemical cycles. As one example, the discovery of the existence and predominance of psychrophilic and mesophilic *Crenarchaeota* below the photic zone in the world's ocean (111, 177, 270) has radically changed our understanding of the distribution of archaea on Earth and raised questions about the function of the *Crenarchaeota* in this global habitat. Further research led to the isolation of representatives of this archaeal clade with the capability of oxidizing ammonium to nitrate (288), the autotrophic microbial process of nitrification that was heretofore known to occur only within the bacterial domain. These studies provided some indication of a potential biogeochemical function for related *Crenarchaeota* in the dark ocean, with significant ramifications for global budgets and cycles of nitrogen and carbon. Another revolutionary discovery emerged from research of deeply buried sediments of the dark ocean, where active microorganisms are now known to persist in sediments hundreds of meters below the ocean floor that are millions of years old (433). These discoveries prompted a recent wave of studies to understand the extent, function, and importance of a deep sedimentary biosphere in the dark ocean (45, 47, 240, 323, 355, 487, 488).

These two examples demonstrate that in addition to their important contribution to human health and disease, microorganisms also dominate dark ocean ecosystems and are relevant to global processes that influence the Earth's environment and, thus, society. Microbes are the principal custodians of the environment, balancing and maintaining Earth's global biogeochemical cycles. Although researchers have recognized for many years the importance of studying the dark ocean, our understanding of this realm has lagged behind that of its sunlit counterparts in the terrestrial and marine realms due to the difficulty in accessing it, as studies in the dark ocean are both technically challenging and expensive. Despite these obstacles, new generations of researchers and experimental tools have emerged, in the last decade in particular, owing to dedicated research programs to explore the dark ocean biosphere. In this review, we summarize the current understanding of microbiology in the dark ocean, outlining salient features of the various habitat types that exist in this vast realm, discussing known (and speculative) types of microbial metabolism and their consequences in global biogeochemical cycling, reviewing patterns of microbial diversity in the dark ocean, and highlighting important new areas of research presently emerging for future study. Before delving into the details of the microbial processes and communities that are characteristic of the various habitats, we present an overview of the classification scheme for dark ocean habitats and a review of the fundamental underpinnings that govern microbial processes in the dark ocean.

DARK OCEAN HABITATS—AN OVERVIEW

The dark ocean can be considered any habitat existing below the photic zone of the ocean. Figure 2 presents a schematic of these different habitats, and photographs in Fig. 3 to 5 highlight some of these. Other than permanent darkness and isolation from photosynthetic pathways as a local means of generating new carbon at the trophic base, another common unifying feature of the dark ocean is relatively high pressures (pressure increases by ~1 atm with every 10 m of water depth). Habitats in the dark ocean span a wide range of temperatures, from relatively cold (some below 0°C) deep-water masses to high-temperature hydrothermal vents (up to 400°C in some places). By volume, low temperatures and high pressures dominate habitable dark ocean environments. Although such conditions are often referred to as "extreme," considering their ubiquity in the environment, these conditions are actually quite average on a global scale.

The body of water below the photic zone in the world's oceans represents the largest water mass on Earth and the largest aqueous habitat for microbial life (Table 1). This water mass blankets the benthic habitats of the dark ocean, serving as a filter between the ocean bottom and the sunlit surface world. The aphotic water column can be highly diverse chemically and physically, ranging from polar to temperate to equatorial conditions and from deep open oceans to shallower seas and containing vertical divisions of water masses based on temperature and salinity. In addition to the water mass above the seafloor, large volumes of water reside and circulate within the basement rocks of the ocean crust—a volume of water referred

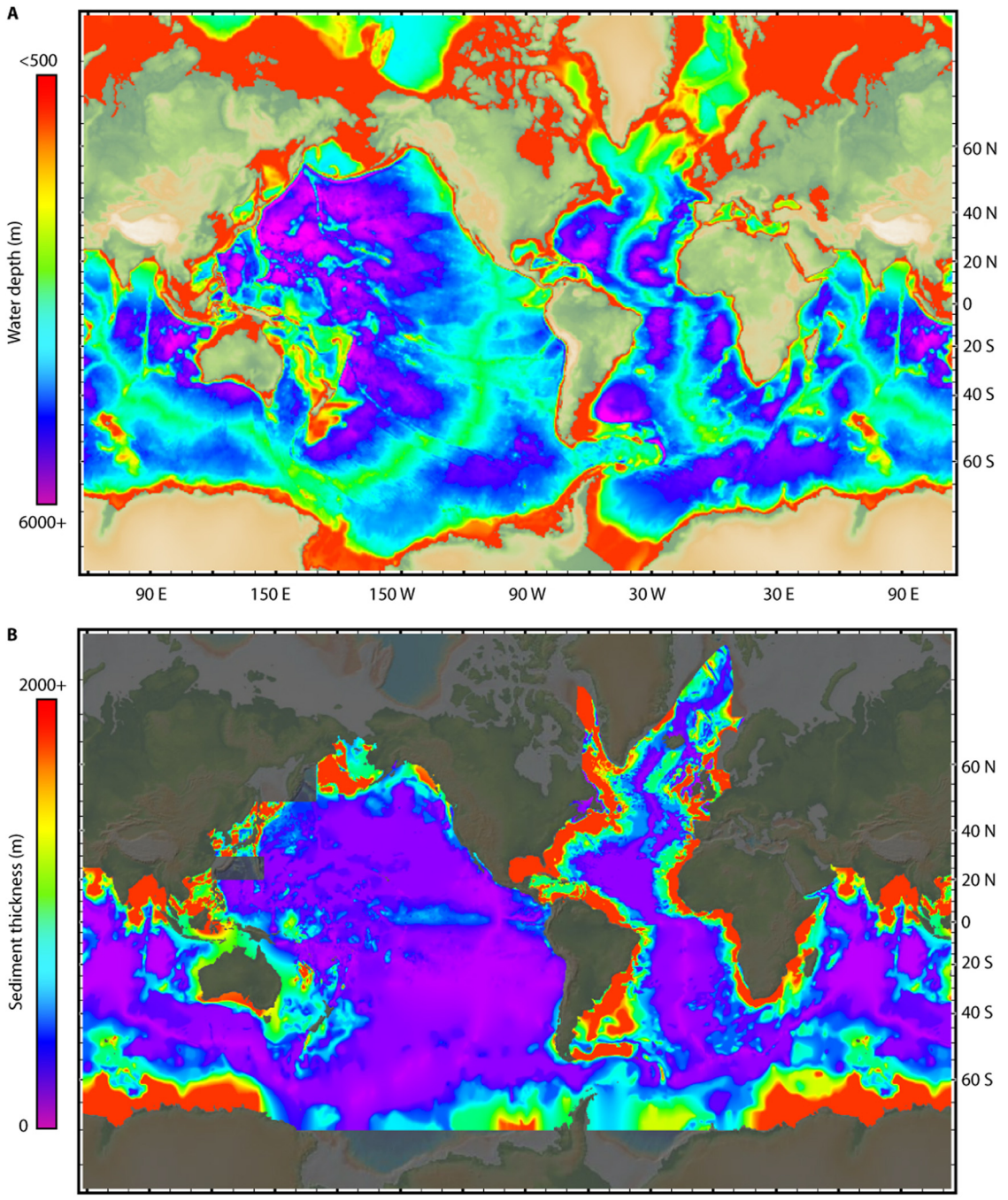

FIG. 1. Global maps of ocean water depth (A) and sediment thickness (B). The water depth scale is from less than 500 m (red) to 6,000+ m (purple). The sediment thickness scale is from 0 m (purple) to >2,000 m (red). Maps were created using GeoMapApp (www.geomapapp.org [474a]).

to as the subseafloor ocean (this moniker does not apply to fluids contained in pore spaces within marine sediments, however, as advective processes typically do not characterize them [see below]). In this subsurface ocean, deep seawater enters into exposed outcrops of the ocean's crust due to thermal and pressure gradients (239, 600, 605), replacing the liquid volume lost as the discharge of hydrothermal fluids. This discharge process occurs as both spatially confined, rapidly advective fluid flow (i.e., as hydrothermal vents along midocean ridges) (Fig. 5F and G) and a cooler, more diffuse emission across large areas of the seafloor (137). The subseafloor ocean harbored in oceanic crust is estimated to circulate the entire volume of the ocean through the crust on the order of every 10^5 to 10^6 years (137, 604), allowing for chemical exchange during fluid-rock interactions that greatly impact many global elemental budgets. Where hydrothermal fluid is discharged into the overlying water column (Fig. 5F and G), mixing of reduced hydrothermal fluid with cool, oxygenated seawater occurs,

TABLE 1. Estimated volumes of various habitats in the dark ocean, with the volume of the ocean photic zone included for reference

Habitat	Vol (m^3)	Reference
Water column (<200 m below sea level)	3.0×10^{16}	608
Water column (200+ m below sea level)	1.3×10^{18}	608
Hydrothermal plumes[a]	7.2×10^{13} (/yr)	43
Subsurface ocean	$\sim 10^{16}$	131
Sediment, all	4.5×10^{17}	283
Shelf sediment	7.5×10^{16}	283
Slope sediment	2×10^{17}	283
Rise sediment	1.5×10^{17}	283
Abyssal sediment	2.5×10^{16}	283
0- to 10-cm layer	3.6×10^{13}	608
Ocean crust[b]	2.3×10^{18}	

[a] The volume of hydrothermal plumes is given as the volume of plume fluid produced per year (adapted from reference 43).
[b] The volume of oceanic crust was assumed by multiplying the average thickness of the oceanic crust (7 km [89]) by the assumed area of seafloor underlain by crust (65% of Earth's surface, or 3.3×10^{14} m^2).

leading to the formation of positively and neutrally buoyant "plumes" of distinct, hydrothermally derived water masses in the water column. Although many chemical compounds precipitate out of the plumes early on, due to the change in temperature or oxidation, chemical signals of hydrothermal input persist in plumes over large spatial areas, many of which are kilometers away from the source venting (185).

At the seafloor below hydrothermal plumes are areas of intense hydrothermal activity, related either to the formation of new ocean crust at midocean ridges and midplate hot spots (Fig. 1 and 2) or to compressional subduction processes occurring where two tectonic plates collide with each other. Hydrothermal activity is also generated in back-arc basins, which derive from a combination of seafloor spreading and subduction processes, therefore producing a unique geological environment (125). The general geological properties of midocean ridges, such as topography and age, depend on whether the ridge is located on a slow-spreading center that tends to have steeper, blockier ridge axes sculpted by tectonic forces or on a fast-spreading axis that is characterized by smoother ridge flanks and well-defined axial valleys at the ridge center (for a more detailed discussion, see reference 492). Off axis from the

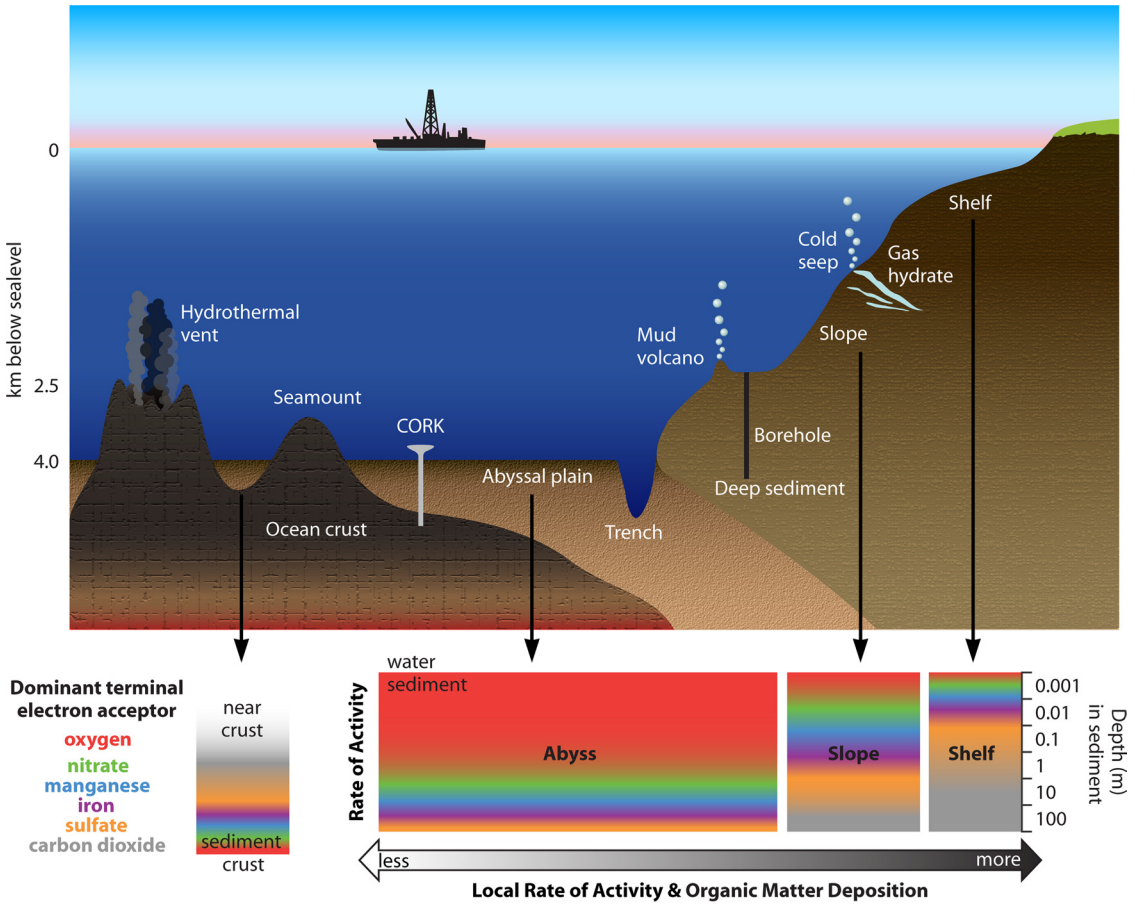

FIG. 2. Schematics depicting a stylized cross section of dark ocean habitats (top; adapted from reference 259 by permission of Macmillan Publishers Ltd., copyright 2007) and representations of sediment biogeochemical zonation (bottom). Note that the upper panel is not drawn to scale. In the lower panel, dominant electron acceptors in the various sediment habitats are indicated by vertical depth into sediment (note the logarithmic sediment depth scale). The relative quantity of organic matter deposited in each sediment type and the scale of metabolic rates in sediment are indicated by the grayscale bar, with dark shades indicating higher rates.

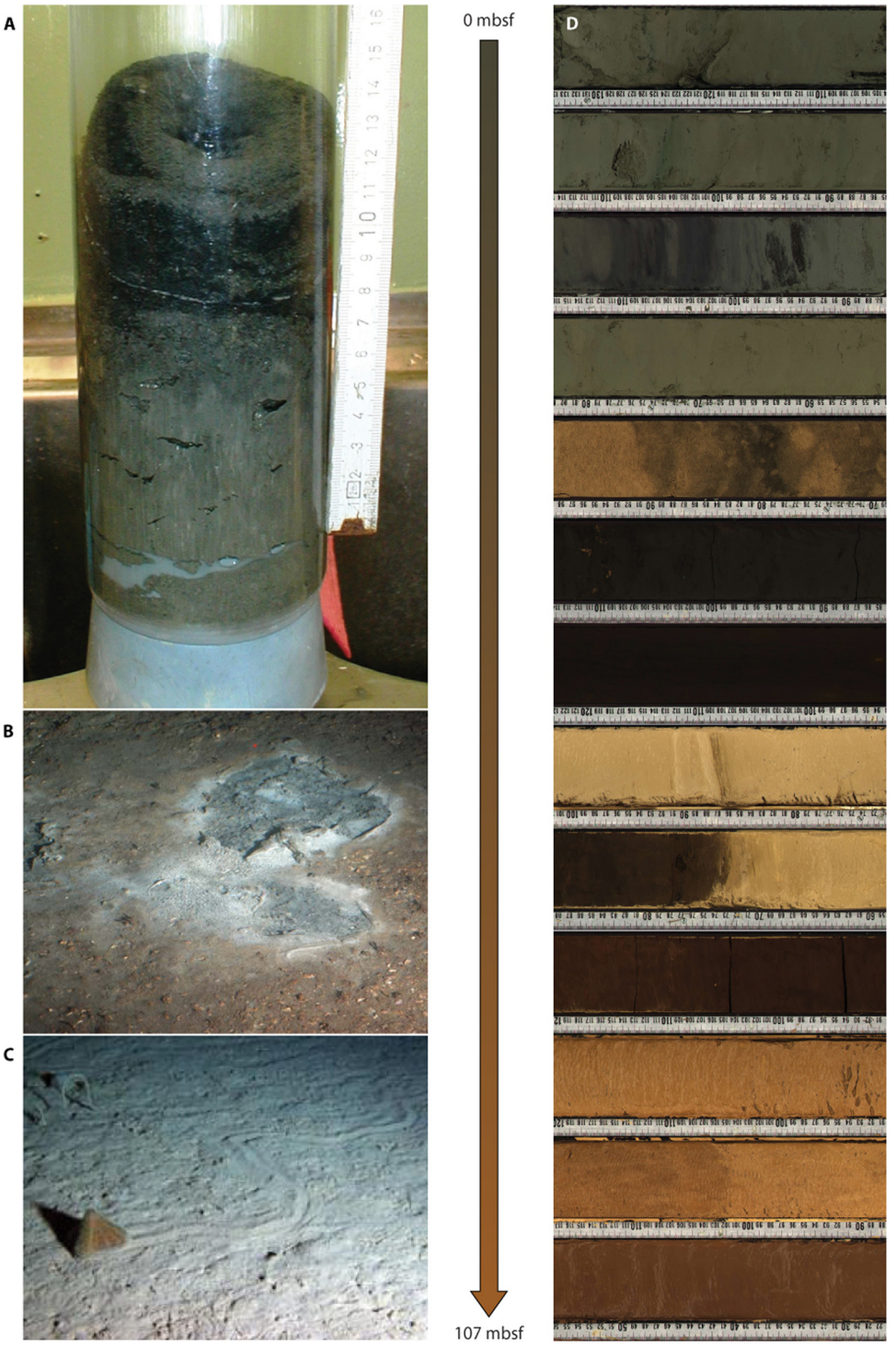

FIG. 3. Photographs of representative sediment habitats in the dark ocean. (A) Surficial sediment from a methane seep in the Black Sea. (B) Mats of sulfur-oxidizing *Beggiatoa* on the sediment surface of the Northwestern Black Sea shelf. (C) Pacific Ocean abyssal plain sediment surface. (D) Compilation of deep sediment layers cored at Hole 1231 (Peru Margin) during Ocean Drilling Program Expedition 201. (Panel A courtesy of T. Treude, IfM Geomar, Kiel, Germany; panel B courtesy of K. Hissmann, JAGO-Team/IfM Geomar, Kiel, Germany; panel C was reprinted with permission of the Monterey Bay Aquarium Research Institute [courtesy of Ken Smith].)

midocean ridges, the ridge flanks subside and become buried by sediment "raining" down through the water column, derived from either surface ocean particulate matter formation or continental inputs. Topographic highs on former ridge axes can protrude from the sediment cover, forming outcrops of oceanic rock basement that are exposed to seawater. In addition, midplate volcanic hot spots can also lead to new crust formation in the form of seamounts (such as the Hawaiian Island chain and

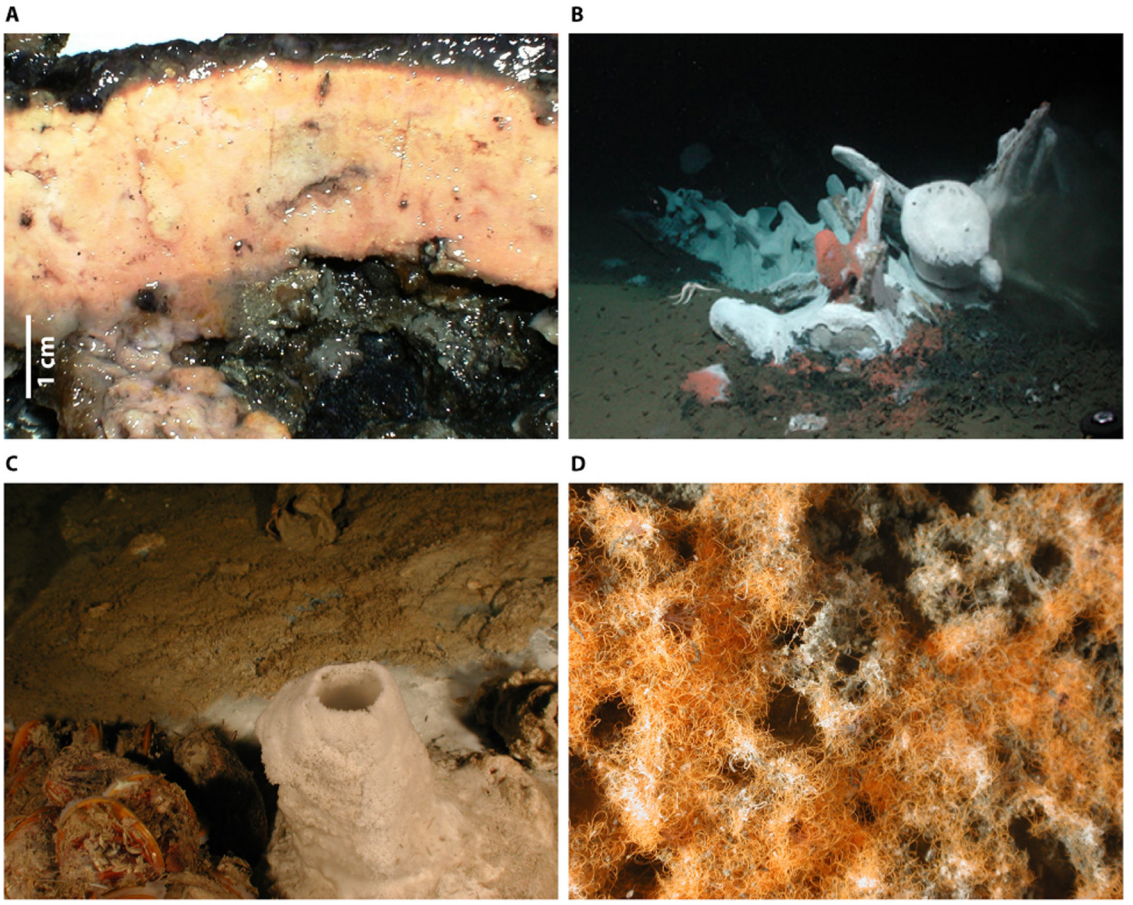

FIG. 4. Photographs of representative marine sediment seep and whale fall habitats. (A) Cross section of a methane-oxidizing microbial mat from a carbonate chimney formed at the seafloor in the anoxic Black Sea. (B) Whale fall ecosystem at the seafloor in the Pacific Ocean. (C) Barite chimney at a mud volcano in the Gulf of Mexico. (D) Close-up image of orange and white *Beggiatoa* bacteria overlying sulfidic sediment at a Gulf of Mexico cold seep. (Panel A courtesy of T. Treude, IfM Geomar, Kiel, Germany; panel B courtesy of Craig Smith, University of Hawaii; panels C and D courtesy of I. R. MacDonald, Florida State University.)

Iceland), which also subside and are partially covered in sediment as the seamounts move off the hot spot. Eventually, oceanic plates are recycled at convergent margins, where one plate subducts beneath another. This process leads to the formation of oceanic trenches between the subducting plate and the convergent margin, which can reach depths of almost 11 km below sea level, as at the Marianas Trench, and to the formation of back-arc volcanic systems on the overriding plate. For more details about the classification of oceanic crust habitats, a thorough review was recently published (491).

The highest deposition rate of sediment to the dark ocean occurs at the continental margin, where terrestrial sediment inputs aggregate with water column particulate formation in the biologically productive continental margin waters. The thickest sedimentary units typically occur on the continental shelf, overlying the contact between continental and oceanic crust. Sediments in these shelf areas can reach depths of >10 km (Fig. 1 and 2). Adjoining the shelf environments is the continental slope, which receives less sediment input, has a steeper gradient than the shelf, and constitutes a transition between the shelf environment and the deeper abyssal plain. Slope failures are known to occur in areas with unstable pressure build-up, leading to the formation of submarine canyons in the slope face and to fans of sediment deposit at the base of the slope in the abyssal plain (577). The abyssal plain is a relatively flat expanse of seafloor sediment overlying oceanic crust, with an average water depth of 4 km. The sedimentation rate over the abyssal plain is generally much lower than the rate closer to shore, and thus the sediments, on average, are shallow, on the order of tens to hundreds of meters thick.

Thick sedimentary sections, occurring in such places as the continental shelves and slopes and at convergent margins above subduction zones, host unique features in the form of mud volcanoes, gas seeps, and gas hydrates (Fig. 4). Mud volcanoes and cold gas seeps are characterized by the emission of fluidized mud and/or gas-charged fluids, resulting from the build-up of pore pressure and/or gas content within sediments. Pressure build-up can be attributed to the movement of buried, neutrally buoyant salt, to sediment slumps, or to gas production from thermal and biological processes. In areas with high gas content, some of the gas can become trapped in gas hydrates, gas-rich ice-like structures formed from the reaction of

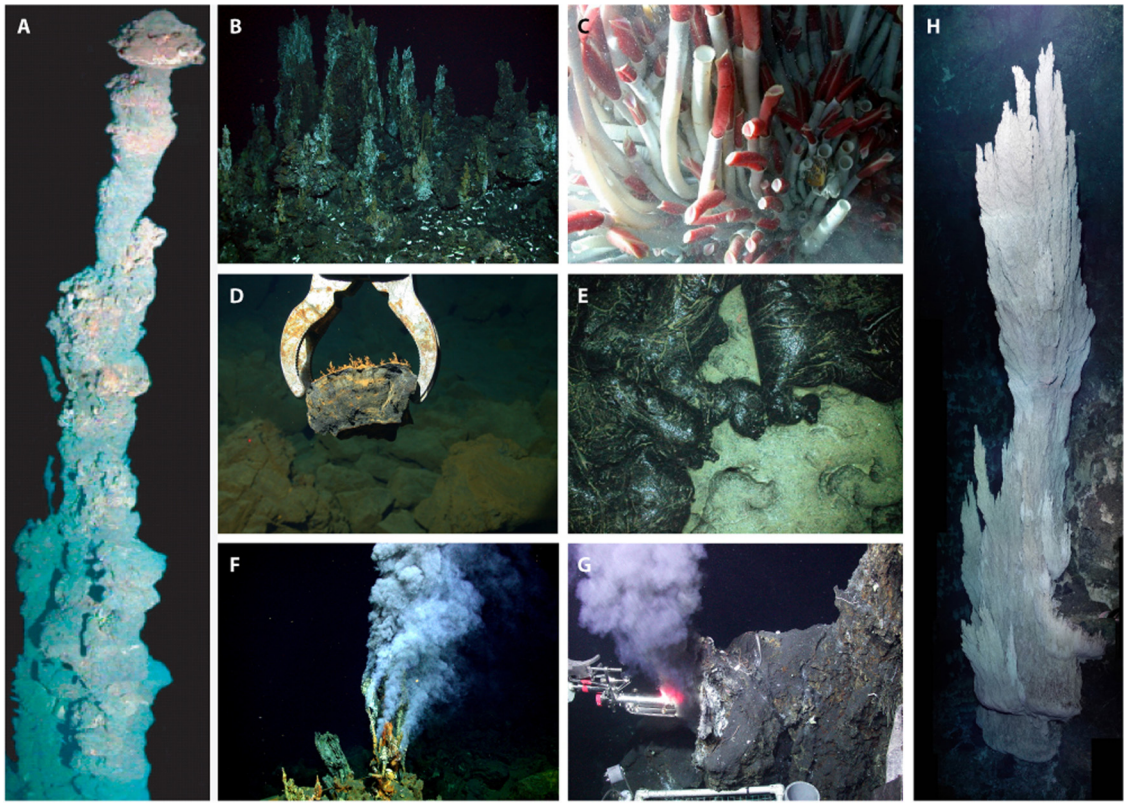

FIG. 5. Photographs of representative oceanic crust and hydrothermal vent habitats in the dark ocean. (A) Nine-meter-tall extinct hydrothermal sulfide chimney off axis of the East Pacific Rise. (B) Active and inactive hydrothermal chimneys at Tu'I Malila vent field, Valu Fa Ridge. (C) *Riftia pachyptila* tube worms at East Pacific Rise. (D) Piece of altered basaltic oceanic crust from the Loihi Seamount being picked up by the ROV *Jason* submersible manipulator. (E) Young basalt flows overlaying older basalt flows at the East Pacific Rise. (F) White smoker hydrothermal chimney at Mariner vent field, Valu Fa Ridge. (G) Black smoker hydrothermal chimney being sampled by the submersible arm of DSV *Alvin* at the Juan de Fuca Ridge. (H) Sixty-meter-tall carbonate chimney at the Lost City hydrothermal vent field. (Panels A, D, and G courtesy of Woods Hole Oceanographic Institution; panels B, C, and F taken with the NDSF ROV *Jason II*, operated by the Woods Hole Oceanographic Institution, courtesy of C. Fisher [PSU] and the National Science Foundation Ridge 2000 program; panel E courtesy of Adam Soule, Woods Hole Oceanographic Institution; panel H courtesy of Deb Kelly.)

gas with water under certain conditions of pressure and temperature (296, 298).

Sediments that occur below the most active surficial depths are often referred to as deep sediment (Fig. 3D) (121, 543, 608). In these sediments, rates of microbial activity and also cell densities are much lower than in the upper sediment layers due to reduced abundances of carbon and energy sources. The upper boundary of the deep biosphere is often operationally defined as somewhere between 10 cm and 10 m of sediment depth (121, 608). Acquisition of sediments below a few meters of sediment depth requires the use of technologically sophisticated (and expensive) oceanic drilling vessels to drill boreholes into the seafloor, akin to drilling a well on land. Ocean drilling has been used for decades in the oil industry and in geoscience research (geology and paleoclimatology), but our ability to explore the existence of life below a few meters in sediments occurred only relatively recently (102, 119, 433). Finally, although macrofauna (fish, sponges, corals, invertebrates, etc.) exist in the dark ocean water column and anchored in sediments and rocks at the seafloor, the deep subsurface is only known to be inhabited by microorganisms.

METABOLIC REACTIONS IN THE DARK OCEAN

To understand the microbiology and ecology of microbial habitats in the dark ocean, it is important to consider how microorganisms utilize substrates and gain energy in these environments. At the surface of the Earth, diverse autotrophic organisms produce energy-rich organic matter by fixing carbon dioxide through photosynthesis, and this organic matter then serves as food for other organisms that convert it back to CO_2 via respiration. These reactions are often closely coupled spatially and functionally, and our ecosystem-level understanding of the biological carbon cycle is based in large part on the balance between these processes. A fundamental distinguishing factor in the dark ocean is that metabolic strategies are based on chemical redox reactions rather than the photosynthetic processes occurring in the sunlit world. Furthermore, respiration pathways and the reactions used for energy generation in the dark ocean are more variably coupled—spatially, temporally, and functionally. In addition to sinking organic matter created via photosynthesis, additional pathways of primary productivity exist in the dark ocean that are often unaccounted for in biogeochemical budgets. Understanding ecolog-

TABLE 2. Common redox reactions and associated standard free energies of reaction that occur in the dark ocean and can be exploited for metabolic energy

Pathway	Reaction	ΔG° (kJ/mol)[a]
Oxic respiration	$CH_2O + O_2 \rightarrow CO_2 + H_2O$	−770
Denitrification	$CH_2O + 4/5NO_3^- \rightarrow 1/5CO_2 + 2/5N_2 + 4/5HCO_3^- + 3/5H_2O$	−463
MnO_2 reduction	$CH_2O + 3CO_2 + H_2O + 2MnO_2 \rightarrow 2Mn^{2+} + 4HCO_3^-$	−557
Fe(III) oxide reduction	$CH_2O + 7CO_2 + 4Fe(OH)_3 \rightarrow 4Fe^{3+} + 8HCO_3^- + 3H_2O$	−697
Sulfate reduction	$CH_2O + 1/2SO_4^{2-} \rightarrow HCO_3^- + 1/2H_2S$	−98
Sulfate reduction (from methane)	$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$	−33
Methanogenesis (from acetate)	$CH_3COOH \rightarrow CH_4 + CO_2$	−24
Methanogenesis (from H_2/CO_2)	$H_2 + 1/4HCO_3^- + 1/4H^+ \rightarrow 1/4CH_4 + 3/4H_2O$	−57
Fermentation (from ethanol)	$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2$	−181
Fermentation (from lactate)	$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COOH + HCO_3^- + 3H_2$	−1,075
Acetogenesis	$H_2 + 1/2CO_3^- + 1/4H^+ \rightarrow 1/4CH_3COO^- + H_2O$	−90
Hydrogen oxidation	$H_2 + 1/2O_2 \rightarrow H_2O$	−263
Methane oxidation	$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$	−859
Sulfide oxidation	$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$	−750
	$H_2S + 8/5NO_3^- \rightarrow SO_4^{2-} + 4/5N_2 + 4/5H_2O + 2/5H^+$	−714
Fe(II) oxidation	$Fe^{2+} + 1/4O_2 + H^+ \rightarrow Fe^{3+} + 1/2H_2O$	−48
	$Fe^{2+} + 1/5NO_3^- + 6/5H^+ \rightarrow Fe^{3+} + 3/5H_2O + 1/10N_2$	−44
	$Fe^{2+} + MnO_2 + 2H^+ \rightarrow Fe^{3+} + MnO + H_2O$	ND
Mn(II) oxidation	$Mn^{2+} + O_2 \rightarrow MnO_2$	−149
	$Mn^{2+} + 2/5NO_3^- + 4/5H_2O \rightarrow MnO_2 + 1/5N_2 + 8/5H^+$	−79
Nitrification	$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$	−302
Anammox	$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$	−345

[a] Values for ΔG° calculations were taken from reference 15. ND, not determined.

ical diversity and microbial metabolic reactions in the dark ocean is a centerpiece of dark ocean research. Furthermore, understanding the principles affecting microbiology in the most extreme or energy-limited environments on Earth—for example, hydrothermal vents or the deep marine subsurface, respectively—provides valuable parameters for constraining the search for life elsewhere in our solar system and for understanding the evolution of life on Earth.

Microbiologists studying life in the dark ocean and the potential for life on other planets are developing key unifying reference frames concerning "habitability" of different environments (491). Energetic constraints that examine the potential of chemical reactions to provide energy for life in the dark provide one such useful construct (27, 223). Approaches that define energetic constraints have been applied in a number of dark ocean habitats (for example, see references 26, 359, and 360) and allow geological and geochemical information to be used for the purpose of predicting which metabolic and physiological processes might characterize dark microbial ecosystems. Such information guides strategies for cultivation and ecological studies as well as for understanding the biogeochemical consequences of dark microbial ecosystems for the global environment.

All life on Earth requires access to sources of energy and carbon. In the absence of light, metabolic energy is harnessed from the coupling of reducing and oxidizing ("redox") reactions (for examples, see Table 2). Energy is obtained when the coupled redox reactions are thermodynamically favorable and yield enough energy for ATP generation. Microorganisms exploit the available chemical energy by developing strategies to overcome the activation energy of reaction and kinetic constraints or by coupling energetically unfavorable reactions with other energy-yielding pathways. Carbon acquisition and energy generation are linked in some cases (i.e., when organic matter is oxidized while another substrate is reduced), but often catabolic and anabolic reactions are separated.

The metabolic activities of microorganisms in the dark ocean depend on the availability and speciation of electron donors (oxidizable compounds) and acceptors (reducable compounds) (174). Distinct zonations of microbial activity based on available electron donors and acceptors are well described and characteristic for marine sediments, which have been studied intensively compared to some other dark ocean habitats. Figure 2 provides a representation of this metabolic zonation in marine sediments, illustrating the typical cascade of usage of preferred electron acceptors and the impact of delivery rates of electron donors (i.e., organic matter) on the gradient of metabolic rates. Although this sequence of usage of electron donors and acceptors is well studied for some environments, recent research indicates that low-level, cryptic biogeochemical cycling may still occur in zones where it is not expected (228).

The availability of metabolic substrates and substrate concentration gradients is determined by diffusive and advective transport processes (for further review, see reference 59). Diffusion, the random movement of molecules, leads to a spread of substances from areas of higher concentrations to areas of lower concentrations, depending on concentration gradients and the speed of diffusion of the molecules, which is typically on the order of 10^{-9} m^2 s^{-1}. In deep ocean water, chemical exchange occurs via the movement of water in currents and along isopycnal gradients, upwelling and downwelling near coastlines and the equator, and mixing with hydrothermal, groundwater, and riverine fluid inputs. Chemical exchange in marine sediments is dictated mostly by molecular diffusion, with advective processes limited to the bioturbation and bioirrigation activities of animals living in surface sediment; to the migration of fluids, fluidized mud, and gasses from deep gas sources or salt deposits; and to the movement of fluids along

faults and fractures in the sediment. In oceanic crust, advective processes include fluid flow through permeable, fractured, and porous hard rock, driven by pressure and temperature gradients. Hydrothermal vents provide conspicuous evidence of crustal fluid flow; however, a large fraction of fluid flow in oceanic crust occurs at lower temperatures (~20°C) and over broad spatial scales (239).

Electron Sources

The dominant electron sources in the dark ocean include organic matter, hydrogen, methane, reduced sulfur compounds, reduced iron and manganese, and ammonium. These electron donors have different abundances and energy potentials and therefore differ in significance as substrates for microbial metabolism. Below, we discuss each of the various electron donors in order, starting from that with the most negative relative reduction potential.

Organic matter. The high redox potential of organic matter compared with most other electron donors (Table 2), coupled with its broad availability, renders it the dominant electron donor in most marine environments, especially in those where it is available in abundance, such as at productive continental margins (Fig. 2). Organic matter is composed of different compounds—simple and complex sugars, proteins, lipids, humics, ligands, organic acids, etc.—each characterized by different reactivities (i.e., susceptibilities to chemical/biological degradation). Thus, utilization of organic compounds as an energy source depends on both the quantity and quality/reactivity of the substrates (58, 212, 308, 330, 418). For example, fresh organic matter is utilized faster than aged organic material in sediments, evidencing the generally more refractory (i.e., resistant to degradation) nature of aged organic matter. Low-molecular-weight compounds are preferentially consumed (22), although the patterns of organic consumption can be affected by external forces such as temperature change (597). Organic material in the dark ocean can be produced *in situ* by extant microorganisms or delivered from a distant source, such as the overlying photic zone or terrestrial sources. In general, terrestrially derived organic matter is more difficult to utilize than water column- and *in situ*-derived organic matter due to differences in material composition (i.e., primarily plant residues versus phytoplankton [32]). Depositional conditions, such as bioturbation activity by benthic macrofauna in sediments, also affect the turnover of organic matter by microorganisms in sediments (418).

In order to become accessible for microbial remineralization (i.e., conversion of organic matter to inorganic carbon dioxide), the particulate and dissolved organic matter in sediments are degraded by hydrolysis and fermentation mediated by both microbial eukaryotes and prokaryotes to form smaller molecules such as short-chain fatty acids, alcohols, and amines. These degradation products are then used by diverse groups of microorganisms and remineralized with various electron acceptors to form carbon dioxide.

Hydrogen. Molecular hydrogen is one of the most energetically favorable electron sources for microorganisms. Therefore, the concentration of hydrogen in the environment is generally very low due to tight competition for this energetic resource. Studies of hydrogen consumption in marine sediments indicate that microorganisms capable of coupling hydrogen oxidation to the reduction of electron acceptors such as metal oxides, sulfate, and carbon dioxide are able to suppress the hydrogen concentration to levels that make the less favorable pathways uncompetitive (222, 339). Hydrogen is also an important intermediate in syntrophic relationships between different groups of microorganisms, a process referred to as interspecies hydrogen transfer (reviewed in reference 569). For example, fermenting microorganisms are often found coupled with methanogens, since methanogenic activity keeps the hydrogen concentration low enough to make the fermentation pathway energetically favorable. Furthermore, hydrogen can also be produced during iron oxidation in sediments, whereby it is rapidly consumed by hydrogenophilic microorganisms (340). Interspecies hydrogen transfer has been speculated to occur in other processes, such as in sulfate-dependent anaerobic methane oxidation (221), but such associations remain controversial (403, 427, 571).

In environments with high hydrogen concentrations, such as in some hydrothermal fluids (particularly in mantle rock-hosted hydrothermal systems), hydrogen oxidation can be a major metabolic pathway. In these systems, hydrogen is produced during serpentinization reactions occurring within ultramafic rocks and can reach millimolar concentrations. It has been speculated for over 50 years that the radiolysis of water to form hydrogen, fueled by the radioactive decay of naturally radioactive elements (i.e., uranium and potassium), may supply this electron donor to organic matter-starved sediment microbes (52, 381), although definitive proof of this mechanism is lacking. The diverse sources and the fast, often immediate turnover of hydrogen by microorganisms in close proximity to the source make it difficult to quantify the overall significance of hydrogen as an electron donor for marine microorganisms, though pursuit of this hypothesis remains active. Technological advances in hydrogen quantification are needed to remedy this gap in knowledge.

Methane. By far, the largest reservoir of methane on Earth occurs buried in marine sediments (607), as dissolved or free gas or in the form of gas hydrate (297). Methane in marine sediments originates from both abiotic and biotic sources. Abiotic methane can be generated in the marine environment either by the thermal degradation of buried organic matter (329) or by the reaction of H_2 and CO_2 during reaction between mafic (i.e., magnesium- and iron-rich) rocks in the oceanic crust through a process known as serpentinization (279). Globally, the largest fraction (80%) (299) of methane in the marine realm is formed biologically from reduction of CO_2 or other low-molecular-weight organic compounds—such as carbon monoxide (CO), acetate (CH_3COOH), formate (COOH), methanol (CH_3OH), methanethiol (CH_3SH), and methylamine (CH_3NH_3)—by methanogenic archaea, as discussed in more detail below.

As an electron donor, microbially mediated methane oxidation occurs with different electron acceptors, with sulfate being quantitatively the dominant electron acceptor for methane oxidation because methane production occurs below the sulfate-rich zone in sediments, and thus sulfate is the first available electron acceptor for methane oxidation. Sulfate-dependent methane oxidation occurs in well-defined zones in anoxic marine sediments, where upwardly diffusing or advecting methane

meets sulfate being delivered to depth from the water column (118, 247). These zones are often referred to as the sulfate-methane transition zone (SMTZ). On a global scale, more than 90% of the potential methane flux from sediments is recycled via microbial methane oxidation before reaching the water-sediment interface (218). Thus, taking into account the magnitude of methane generated in marine sediments (218, 460), microbial methane oxidation is one of the most important controls on greenhouse gas emission and climate on Earth. At cold seeps, the upward flux of methane is so high that it is not entirely oxidized in the SMTZ but reaches the sediment surface and water column. At these sites, methane may be oxidized by other electron acceptors besides sulfate, such as oxygen. Aerobic methane oxidation is a chemosynthetic energy source within some symbiont-containing animals that reside at gas seeps and hydrothermal vents (80, 81, 126). Additionally, aerobic methane oxidation also occurs at hydrothermal vents where aerobic seawater mixes with methane-rich hydrothermal fluids (106, 416, 417, 570, 587). Nitrite-dependent methane oxidation has been observed in freshwater sediments and enrichment cultures (149, 151, 452) and could theoretically occur in cold seep sediments, although the discovery of these processes is so recent that their existence in marine habitats has not yet been well documented. In marine sediments, methane is usually depleted by sulfate reduction before it reaches nitrite-containing sediments, thus limiting significant rates of nitrate-dependent methane oxidation to environments with relatively high nitrate and low sulfate concentrations. Based on thermodynamic energy yields, methane oxidation could theoretically also be coupled to metal (i.e., iron and manganese) reduction, and some preliminary evidence suggests that this process does occur (39), but its global significance has not been demonstrated.

Reduced sulfur compounds. Many reduced sulfur compounds (e.g., sulfides)—such as elemental sulfur (S^0), hydrogen sulfide (H_2S), methanethiol (CH_3SH), dimethylsulfide [$(CH_3)_2S$], pyrrhotite ($Fe_{1-x}S$), pyrite (FeS_2), chalcopyrite (CuS), and sphaelerite (ZnS)—have been demonstrated to be used as electron donors by microorganisms. Hydrothermal vent fields hosted within basaltic crust are often rich in sulfide deposits due to the precipitation of particulates from H_2S and/or metal-rich fluids that mix with cold oxygenated seawater. Sediments can have significant levels of H_2S, and sometimes iron sulfides and S^0, due to the generation of sulfide from sulfate reduction.

In sedimentary environments and at hydrothermal systems, H_2S and S^0 produced by microbial sulfate reduction or geothermal processes are oxidized by a number of electron acceptors, including oxygen, nitrate, and metal oxides [i.e., Fe(III) and Mn(IV) oxides], through both abiotic and microbially mediated processes (16, 57, 127, 262, 269). Anaerobic H_2S oxidation with iron oxides, manganese oxides, and nitrate also consumes some sulfide in marine sediments (168). In sediments with high H_2S fluxes, allowing H_2S to reach the sediment surface, both free-living and symbiotic bacteria gain energy from sulfide oxidation with nitrate (see "Marine Sediments"). Other sulfide oxidizers reside as chemosynthetic symbionts in mussels, clams, snails, and polychaete worms (Fig. 5C) (126, 406) and use oxygen as an electron acceptor. S^0 can be oxidized microbially through both disproportionation and oxidation reactions to H_2S with several electron acceptors, including Fe(III) oxides and nitrate (16, 156). In sediments and at sulfur-rich hydrothermal vents, free H_2S reacts, both biotically and abiotically, with reduced iron to form pyrite (490), which can serve as another source of energy for microorganisms in both oxic and anoxic environments (130).

Reduced iron compounds. Reduced iron [i.e., Fe(II)-bearing species such as aqueous Fe^{2+} and mineral-bound forms such as pyrite (FeS_2)] can also be used as an electron donor in dark ocean habitats. In the open ocean, Fe(II) derives from alteration reactions occurring between crustal materials and fluids in hydrothermal settings, whereas in sediments, Fe(II) derives mainly from deposition and from anaerobic reduction of iron oxides. In marine sediments, the soluble ferrous iron migrates, via diffusive and advective processes, into zones with suitable oxidizing species—oxygen, nitrate, and manganese oxides—which can be coupled to metabolic reactions (Table 2). Fe(II) also diffuses to where it reacts with reduced sulfur species to form iron sulfides, such as pyrite, which can also be used as electron donors, although some empirical evidence suggests that this process may not be important for microorganisms in marine sediments (486). A microbiological role for Fe oxidation in marine sediments has been studied in only a few cases (486), as it was previously assumed that life could not exploit Fe oxidation reactions with rapid abiotic kinetics under aerobic conditions at circumneutral pH.

Recent evidence shows that microorganisms are responsible for Fe oxidation in some hydrothermally derived mineral substrates under cold, microaerobic conditions (144, 145), when abiotic kinetics are more sluggish, indicating that microbial Fe oxidation may be more prevalent than previously assumed. Hydrothermal iron inputs to the global deep ocean rival what is delivered to the oceans from riverine sources (605). Similarly, Fe that is released from rock and minerals in the deep ocean during oxidative alteration has been shown to support activities of Fe-oxidizing bacteria (26, 132–134). Fe(II) is the most abundant reduced element in Earth's crust and makes up 7 wt%, on average, of the elemental abundance of the ocean crust underlying the oceans (26); consequently, reactions between oxygen and Fe are commonly catalyzed by microbes, although this process is poorly studied. Even less well understood are microbially mediated reactions in the ocean between Fe(II) and nitrate, as there are conflicting reports of their occurrence (238, 516), or with manganese oxides, as there are few data available.

Ammonium. Ammonium is the most reduced form of nitrogen and an important electron donor in the dark ocean. In most of the ocean, ammonium derives from the breakdown of nitrogen-containing organic matter by either biological degradation or hydrothermal alteration. At smaller quantities, ammonium is also generated via a microbial process referred to as dissimilatory nitrate reduction (62). Some recent studies indicate that microbial nitrogen fixation, whereby dinitrogen gas is converted into ammonium, is another source of ammonium in the dark ocean, as in the case of nitrogen fixation linked to the anaerobic oxidation of methane or sulfate reduction in marine sediments (44, 107). Other genomic surveys indicate the presence of nitrogen-fixing microorganisms inhabiting the surface of seafloor-exposed basaltic crustal rocks (354). These findings indicate that nitrogen fixation may contribute more ammo-

nium to the marine benthic ecosystem than previously thought, although the quantitative importance of this process is not well documented.

There are two main metabolic reactions involving ammonium consumption: ammonium oxidation with oxygen (nitrification) and ammonium oxidation coupled to the reduction of nitrite (known as the anammox process [*an*aerobic *amm*onium *ox*idation]) (Table 2). Ammonium oxidation with oxygen has been recognized for some time in some alpha- and gammaproteobacteria, but it was recognized only recently that some groups of autotrophic *Crenarchaeota* also mediate nitrification (288). These *Crenarchaeota* are numerically dominant in some dark ocean habitats, such as the lower water column and oxic surficial sediments (173), and their discovery has renewed interest in understanding the global extent of ammonium oxidation and primary production in the dark ocean by archaea (245). Anammox is another relatively recent discovery, first observed in a wastewater fluidized bed reactor in 1995 (387). Subsequently, anammox has been observed in microaerobic and anaerobic habitats of the dark ocean, including sediments and the marine water column (489). During anammox, ammonium reacts with nitrite to form dinitrogen gas, thus making this process a sink of fixed, bioavailable nitrogen. Anammox may account for 25 to 30% of the removal of fixed nitrogen from marine systems, representing a globally important fixed N sink (62).

Other electron donors. Although less well studied, other electron donors in the dark ocean include methylated compounds (such as methanol, methylamines, and methyl sulfides such as methanethiol and dimethylsulfide) and carbon monoxide gas (CO). Practically all intermediate products of oxidation pathways may serve as electron donors as well, but since it is often difficult to discriminate partial pathways from the overall pathway without targeted *in vitro* experiments, the role that such pathways play in the environment is not very well constrained. However, cycling of these other compounds may provide other energy sources supporting microbial diversity in dark ocean habitats, especially for the rarer members of a community, so understanding their sources and sinks in the environment is relevant.

Electron Sinks

The common electron acceptors that are available in the dark ocean, in order by highest electron accepting potential (Fig. 2; Table 2), include oxygen (transported via global seawater circulation from the photic zone, where it is produced by photosynthesis), nitrate (NO_3^-; mainly formed by nitrification of ammonium produced in the degradation of organic matter), nitrite (NO_2^-; typically generated from either nitrate or ammonium), manganese and iron oxides (derived from hydrothermal, riverine, and dust inputs to the ocean), oxidized sulfur compounds (sulfate, mainly derived from continental erosion, and elemental sulfur and thiosulfate, derived from reoxidation of H_2S), and carbon dioxide (sourced from organic matter degradation, air-sea gas exchange, and hydrothermal and serpentinization inputs). In addition, halogenated organic compounds are potentially important electron acceptors in the dark ocean, especially in anaerobic subsurface sediments (180), although such dehalogenation reactions are very understudied.

The preferred utilization of electron acceptors in the environment tends to follow thermodynamic energy yield, where electron acceptors with the highest redox potential versus a given electron donor are consumed first (Fig. 2). The sequential reduction of electron acceptors has been shown most extensively in marine sediments, where electron acceptors with higher redox potential are consumed shallower in the sediment (174), although the principle also applies in other dark ocean habitats. In general, oxygen is the preferred terminal electron acceptor in sediments, followed by nitrate, Mn(IV) species, Fe(III) compounds, sulfate, and finally carbon dioxide (87, 174). The contribution of each electron acceptor to the overall metabolic activity in any environment is dependent in part on the availability of that electron acceptor. For example, in marine shelf sediments, nitrate reduction yields more energy than sulfate reduction and is therefore consumed preferentially, but the majority of organic matter degradation is coupled to sulfate reduction, as sulfate exists in much higher concentrations than those of nitrate in seawater (256).

Oxygen. Oxygen has the highest redox potential of the electron acceptors. It is produced in the photic zone through the activity of oxygenic photosynthesis and then transported to depth in the ocean via mixing (ocean circulation). In the dark ocean water column, oxygen is the dominant electron acceptor for metabolic reactions, except in areas where it is depleted (oxygen minimum zones). In marine sediments with moderate to high concentrations of organic matter, oxygen is consumed over the first few millimeters to centimeters of depth into the sediments, whereas in organic-poor sediments, oxygen can persist for meters—down to oceanic crust in some places (121). In cool regions of oceanic crust, oxygen is carried in circulating/recharging fluids and may serve as an electron acceptor for oxidizing reduced iron, manganese, and sulfide existing within igneous ocean crust (26). Oxygen is typically absent in discharging warm hydrothermal fluids (due to low solubility and reactions between seawater and rock at elevated temperatures); however, reactions with oxygen are possible in the zones of mixing of hydrothermal inputs with seawater (recharge zones). In addition to aerobic respiration of organic matter in sediments, all other electron donors are also eventually reoxidized by oxygen (Table 2), and thus the oxygen consumption rate is a measure of the overall microbial activity in sediments (193).

Nitrate and nitrite. Nitrate in the dark ocean originates from the nitrification of ammonium, which is the product of either organic matter degradation (i.e., ammonification) or nitrogen fixation. Nitrate can be reduced to nitrite via the process of dissimilatory nitrate reduction, or nitrate can be reduced to the gases nitric oxide (NO), nitrous oxide (N_2O), and/or dinitrogen (N_2) during denitrification (62). As mentioned before, nitrite is also used in the anammox process in anaerobic marine sediments, where it oxidizes ammonium. Nitrate reduction coupled to sulfide (H_2S) oxidation is known to occur in sedimentary systems (167, 496), and nitrate reduction may also be coupled to the oxidation of mineral sulfides in sediments and within oceanic crust (130, 131, 134). Nitrate or nitrite reduction coupled to methane oxidation has been suggested to occur in some

marine sediments (150), although the global existence of this process is not well documented.

Manganese and iron oxides. Solid oxides of manganese and iron also play an important role in redox geochemistry in the dark ocean, particularly in marine sediments. Processes involving these metal oxides have been recognized for many decades; however, until the late 1980s, there were no known microbiological processes that could explain the harnessing of metabolic energy from solid forms of metal oxides. Since then, major studies have been conducted to examine dissimilatory metal-reducing microorganisms that can use both aqueous and solid substrates (as reviewed in references 338, 404, and 405). The mechanisms by which microorganisms deliver electrons to the solid metal oxides during respiration vary, with some microbes using enzymatic pathways at the cell wall, others using extracellular electron shuttles, and still others perhaps transferring electrons via conductive "nanowire" appendages (198, 338, 405, 410, 461).

As noted above, delivery of metal oxides to the ocean can occur via many external inputs (dust, rivers, and hydrothermal vents). They can also accumulate via diagenetic processes in and on marine sediments (i.e., ferromanganous crusts and nodules). In marine sediments, the reduced, aqueous forms of manganese and iron are generally observed at very low concentrations until below the zone of nitrate depletion. Below the nitrate reduction zone and before the zone of sulfate depletion, reduced manganese and then iron concentrations increase, reflecting metal oxide reduction. The aqueous reduced products diffuse away from the zone of production until they are either reoxidized in shallower sediments or precipitated with sulfide generated from sulfate reduction at deeper regions. Oxides of manganese and iron are also generated during the mixing of hydrothermal fluids with cold oxygenated seawater, and these oxides can also fuel microbial metabolism in hydrothermal habitats (362).

Oxidized sulfur compounds (sulfate, sulfite, elemental sulfur, and thiosulfate). Sulfate is by far the most abundant oxidized sulfur species in the dark ocean, with an average concentration in deep ocean waters of 28 mM. Sulfite, elemental sulfur, and thiosulfate can also be used as electron acceptors. These species are often derived from oxidation reactions of H_2S and can be involved in relatively rapid disproportionation reactions, where they are simultaneously reduced and oxidized to form two different chemical species (54, 156). Substrates for sulfate-reducing bacteria can include low-molecular-weight organic compounds (i.e., short-chain fatty acids and some lipids) and hydrogen, but some sulfate reduction in marine sediments is also linked to the anaerobic oxidation of methane and other hydrocarbons (256, 263, 264). Although sulfate is abundant in the dark ocean water column and in some of the cooler fluids circulating through oceanic crust, sulfate reduction is not a major process in these habitats due to the inhibitory presence of oxygen on sulfate-reducing microorganisms, although the process could occur in an anaerobic microniche. Based on stable isotope data, sulfate reduction has been inferred in 50-million-year-old ocean crust obtained through ocean drilling (474), but the extent of sulfate reduction in this habitat is unclear. Sulfate reduction also occurs at hydrothermal vents, as indicated by the isolation of several thermophilic sulfate reducers from these environments (251, 385), although their presence, based on 16S rRNA gene surveys, is generally low.

Carbon dioxide. Carbon dioxide is the final oxidized carbon product of organic matter degradation and remineralization. It can also be formed during serpentinization reactions in ultramafic oceanic crust. Carbon dioxide exists in pH-dependent equilibrium with bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions in solution. Microorganisms consume carbon dioxide during methanogenesis with hydrogen as an electron donor, and it can also be used to fuel autotrophic metabolism such as acetogenesis. A build-up of carbonate in sediment pore waters also leads to oversaturation and the subsequent precipitation of authigenic carbonate minerals (calcium and magnesium carbonates) within sediments. In some exceptional examples, massive carbonate precipitation leads to the formation of submarine carbonate crusts, platforms, and seamounts (294, 368, 445).

Other Microbial Processes in the Dark Ocean

Although less significant in terms of quantitative support of microbial metabolism in the dark ocean, many other microbially mediated processes occur which are relevant both in terms of global chemical budgets and as cornerstones for supporting diversity. In some cases, these reactions are associated with secondary energy-generating pathways during periods of starvation from the typically used substrates. For example, during anaerobic periods that starve sedimentary giant sulfide-oxidizing bacteria of an electron acceptor, stored intracellular polyphosphate is sacrificed to provide energy for the uptake of acetate (497). This anaerobic release of phosphate from such bacteria appears to coincide with massive depositions of the mineral phosphorite in marine sediments (497), and thus it may significantly impact the global phosphorus budget. As another example, microbially mediated nitrogen fixation in the dark ocean is an energy-demanding process, yet it may be an important pathway for the delivery of fixed bioavailable nitrogen to dark ocean microbial communities. Recent discoveries of nitrogen fixation associated with sediment sulfate reduction and methane cycling suggest that the potential for this pathway may be broader than previously assumed (44, 107). Such microbially mediated processes may support some of the microbial diversity observed in dark ocean habitats. Furthermore, from theoretical calculations of energy yields, there are other redox reactions that could potentially be used for energy generation by environmental microorganisms in the dark ocean (e.g., H_2, H_2S, or CH_4 oxidation with nitrite or oxidation of CO with various electron acceptors), but they have not yet been considered and looked for in nature.

DOMINANT REACTIONS AND MICROORGANISMS IN DARK OCEAN HABITATS

It is worth noting that our understanding of processes and microbial communities in the dark ocean are often reliant on "snapshots" of the environments due to the episodic nature of oceanographic research, particularly in remote realms. Thus, a system is often studied only during a specific period, depending on when a research vessel can visit that particular site. "Well-studied" systems are those that have received numerous visits,

FIG. 6. Photographs of commonly used sampling tools for dark ocean research. (A) ROV *Jason II*, Woods Hole Oceanographic Institution. (B) ROV/AUV *Nereus*, Woods Hole Oceanographic Institution. (C) Rosette of Niskin water sampling bottles and a CTD (conductivity, temperature, depth) sensor package. (D) Multicoring device for collecting surficial sediments. (E) Scientific ocean drilling vessel RV *JOIDES Resolution* for collecting deep sediments and hard rock. (F) View from the inside of the *Johnson SeaLink* submersible, Harbor Branch Oceanographic Institute, working at a cold seep in the Gulf of Mexico. (G) Launch of the *Johnson SeaLink* submersible, Harbor Branch Oceanographic Institute. (Panels A and C courtesy of J. Sylvan; panel B courtesy of Robert Elder, copyright the Woods Hole Oceanographic Institution; panels D and G courtesy of B. Orcutt; panel E courtesy of William Crawford, IODP/TAMU; panel F courtesy of I. R. MacDonald.)

but these studies still represent snapshots in time that must be weaved together to develop temporal patterns. In the past decades, the use of observatories at representative sites has augmented our ability to monitor and sample the habitats of interest over time. Observatories include chemical and physical sensors, *in situ* experimentation devices, time-lapse cameras, and other simple and sophisticated devices to monitor changes in the system under investigation or to actively perturb a system to monitor the response. The use of observatories in the dark ocean is highlighted later in this review. The following sections summarize the current state of knowledge about the various types of habitats in the dark ocean, starting with the open ocean habitat,

then discussing marine sediments and ocean crust, and ending with hydrothermal vent-related environments. For reference, Fig. 6 displays some of the common sampling tools and platforms used to acquire samples from the dark ocean.

The Aphotic Pelagic Ocean

General physical and chemical parameters. Microbial life in the pelagic ocean in the photic zone (0 to 200 m) is quite different from life in the mesopelagic (200 to 1,000 m) and bathypelagic (>1,000 m) zones (Fig. 2). The most obvious difference is the presence of light, which is absent below 200 m (on a global average), leaving all organisms that reside in the mesopelagic and bathypelagic zones in perpetual darkness. Phytoplankton, which are light-dependent primary producers, are absent below 200 m; protists, bacteria, and archaea dominate ocean water at depths of >200 m. While inorganic nutrients (NO_3^+, PO_4^{3-}, SiO_3^-, and metals that behave like nutrients, such as Fe, Cd, and others) are nearly absent in the photic zone, they are remineralized and released into the water by microbial activity below the photic zone, leading to a relative abundance of these nutrients below 200 m. The water is also colder in the absence of the sun's influence; the mesopelagic zone ranges from 20°C down to 4°C, and typical bathypelagic water is about 2 to 4°C. Local currents and turbulence are less intense below the photic zone because of a lack of surface wind effects. The currents that predominate are in the form of major water masses moving via ocean circulation along the oceanic conveyer belt (67) at all depths in the ocean.

Pressure is another variable factor between the photic zone and the aphotic water column. Considering that pressure increases by roughly 10 MPa/km of depth in the ocean, at a mean ocean depth of 4 km, the bottom of the ocean experiences a pressure of nearly 40 MPa. Roughly 70% of the ocean floor is at high pressure (1). A detailed description of the biological consequences of life under high pressure is outside the scope of this review, but interested readers can consult an excellent review on the subject elsewhere (36). Briefly, piezophiles—organisms that thrive at high pressure—are often phylogenetically related to microorganisms from cold, deep water, suggesting that piezophily arose from previously cold-adapted species instead of from shallow water species that first adapted to cold and then later to high pressure (307). Investigations of piezophily are currently experiencing a renaissance, primarily due to technological improvements in collection and incubation of deep dark ocean samples (for example, see references 435 and 531). Especially for high temperatures, these studies have revealed important observations on the effects of sustained high pressure and temperature on microbial activity. For example, a piezophilic *Methanopyrus* species was observed to have drastically different carbon fractionation factors when incubated at high pressure and temperature versus high temperature and only moderate pressure (531). Such observations may challenge common assumptions used in previous studies of stable carbon isotope fractionation for determining zones and rates of microbial activity.

There is more biomass in the photic zone, since this is where the majority of marine autotrophy takes place, whereas below the photic zone, biomass decreases in concentration by about 2 orders of magnitude (204, 391). Prokaryotic biomass in the dark pelagic zones is often heterogeneously distributed on the small scale, characterized by marine snow particles—aggregations of particulate organic matter (POM) that host higher densities of microorganisms living in a biofilm around the sinking particulate matter (7, 549). The particulate matter in marine snow can consist of POM (such as fecal pellets and exopolymeric substances exuded by prokaryotes and eukaryotes), dust particles, and heterotrophic prokaryotes that aggregate as a suspended biofilm. Larger fragments of particulate matter also exist in the form of sinking mucous sheaths or webs produced and shed by some animals within the water column (7), although relatively little is known about the ecology of this sinking matter due to its ephemeral nature.

Despite these differences between the euphotic and aphotic ocean zones, particularly with respect to energetic regimens, the mesopelagic and bathypelagic zones are far from a biotic desert and are not static as once thought (19).

Metabolic reactions. Oxygen is relatively abundant in most portions of the mesopelagic and bathypelagic zones, and oxygen-fueled respiratory metabolic pathways predominate. Water column profiles of dissolved oxygen concentrations display minimums in the thermocline (water between 200 and 1,000 m, where the temperature decreases rapidly) due to high rates of aerobic organic matter degradation by microorganisms in this zone. Water masses below the thermocline are typically replenished by deeper oxygenated waters traveling along the oceanic conveyor belt (67, 69); the formation of ice at high latitudes leaves behind colder, saltier, and denser seawater which sinks below the warmer water masses before its oxygen is utilized. Conservation of mass in the oceans dictates that the sinking water sets in motion the conveyor belt noted above, which travels throughout the world's oceans, below the thermocline.

In general, rates of aerobic heterotrophic prokaryotic production (as measured by either leucine or thymidine incorporation, indicating protein production or cell division rates, respectively) exhibit the same trends as cell abundance densities (391, 463). That is, the volumetric rate of heterotrophy observed in any parcel of water is dependent on the density of prokaryotes in that parcel of water. While distributions and heterotrophic production rates of deep ocean prokaryotic communities are well established (18), uncertainties exist regarding the factors that explain the observed trends. One possible factor is the availability of particulate organic carbon (POC) versus dissolved organic carbon (DOC) (20, 68, 203, 204, 391). Overall, it appears that the majority of heterotrophic respiration in the dark ocean is fueled by organic matter delivered from sinking particles (POC) and less from direct DOC export from the surface ocean (18, 20). The factors determining growth rates of deep-water prokaryotic communities are also unclear. For instance, one study in the Pacific Ocean found turnover times of 0.5 to 2.2 years at 1,000 m and 2.6 to 17.9 years at 1,000 to 4,000 m (391), while another from the North Atlantic measured no turnover times longer than 0.167 year anywhere in the water column (463). More studies are required to determine the fundamental factors driving microbial abundance, activity, and residence times in the dark pelagic ocean.

Primary production also occurs in the dark ocean, in addition to the heterotrophic production described above. Whereas

it was typically assumed that primary production occurred only in the sunlit surface ocean, recent studies suggest that roughly one-third of global ocean primary production occurs in the aphotic pelagic ocean (20, 110), and another study suggests that autotrophy may represent >80% of the archaeal productivity in the mesopelagic zones (245). One of the sources of this autotrophy in the deep ocean is likely nitrification, the conversion of ammonium to nitrite, which is carried out by beta- and gammaproteobacteria as well as *Crenarchaeota* (173, 374, 618). At present, it is unclear which of these microbial groups plays the largest role in aerobic ammonium oxidation in the deep water column, and this remains an active area of research (2), but strong evidence indicates that it is likely dominated by the *Crenarchaeota*, at least some of which exhibit extremely high affinities and low K_m for ammonia, very much in line with the ammonia concentrations measured in the deep ocean (350). Other autotrophic carbon sources in the deep water column include anaerobic and microaerophilic metabolism occurring within microniches on sinking particles, where prokaryotes are the primary degraders of the sinking organic matter (84).

Other metabolic pathways have been detected in the aphotic pelagic ocean, although whether they contribute to autotrophic or heterotrophic production is currently speculative (19). For example, a number of studies have observed the presence of microbes in the deeper water column possessing the capability for carbon monoxide oxidation (113, 352, 575). While CO oxidation is theoretically feasible as a source of energy to drive autotrophy, the concentrations of CO in the ocean are too low to support the high K_m required by the enzymes involved (380). In the surface ocean, it is hypothesized that the microorganisms capable of CO oxidation, such as the *Roseobacter* clade of the *Alphaproteobacteria*, use this process as a supplemental energy source, managing to consume the majority (86%) of the CO produced daily (380). Genes for CO oxidation associated with the *Chloroflexi* and *Bacteroidetes* phyla have been detected in deep waters, suggesting the potential for this process outside the photic zone (351).

Additionally, in low-oxygen environments of the aphotic water column, the observation of *Planctomycetes* bacteria indicates that anammox may be another metabolism supporting primary production in this environment (295, 614). For example, anammox was found to be the predominant nitrogen removal process in the oxygen minimum zone of the Benguela upwelling system off the coast of southwest Africa (295). However, denitrification, a heterotrophic process, was found to dominate in the oxygen minimum zone in the Arabian Sea (588). The factors explaining the dominance of anammox versus denitrification in the removal of fixed nitrogen are currently unclear (304).

Microbial distribution and diversity. Prokaryotic abundance—the sum of bacterial and archaeal cells assayed via DNA-based stains—in the aphotic pelagic ocean typically decreases by 1 to 2 orders of magnitude from that in surface waters: cell densities of roughly 10^5 to 10^6 cells ml^{-1} in surface waters decrease to roughly 10^3 to 10^5 cells ml^{-1} at depth (391, 438, 463). In the aphotic pelagic ocean, it appears that free-living cells are proportionally more abundant than particulate attached cells (84). A marked increase in cell density may occur near the bottom of the water column, within a few hundred meters above sediment, resulting from fluxes of nutrients from the benthos (391). Although cell abundances may decrease with depth in the water column, recent evidence suggests that microbial diversity increases with depth (see below).

Based on recent molecular ecological and genomic studies conducted in multiple ocean basins (37, 70, 92, 113, 214, 216, 270, 382, 444), it is now clear that microbial communities are stratified with depth in the pelagic ocean, as recently reviewed (19). Fingerprinting techniques that generate visual markers of microbial diversity based on a gene of interest, such as automated ribosomal intergenic spacer analysis (ARISA) (161) and terminal restriction fragment length polymorphism (TRFLP) analysis (325), are useful tools for relative comparisons of microbial communities in different samples. For example, ARISA with water samples taken at 500 m, 1,000 m, and 3,000 m from different oceans revealed that microbial communities from the same depths in different oceans were more similar than those from the same ocean but different depths (216). Although this depth-dependent trend may be apparent for dominant microbial species or groups within a community, the general adage that "everything is everywhere but the environment selects" (25) may still be valid, as the methods used to determine stratification patterns are likely biased against rarely occurring members of a microbial community. Recent application of massively parallel sequencing of fragments of the 16S rRNA gene in marine samples documented a rare biosphere of infrequently occurring microbial species (505), which may represent a "seed community" of microbes that can adapt to changing environmental conditions. Using similar sequencing methods, other researchers have demonstrated that the rare biosphere also displays patterns, suggesting an active response to environmental variables (70, 181). Determining the potential and dynamics of the rare biosphere in the dark ocean requires more investigation.

Both archaea and bacteria exhibit global patterns in vertical stratification in the dark ocean water column. A general trend that has been observed in multiple ocean basins is the increase with water depth of the proportion of archaea in the microbial community (214, 270, 613). Marine group I *Crenarchaeota*—whose cultured members include the ammonium-oxidizing species "*Candidatus* Nitrosopumilis maritimus" (288) and the mixotrophic sponge symbiont *Cenarchaeum symbiosum* (200)—are ubiquitous in aphotic waters (172, 176, 357). In the North Pacific Gyre, *Crenarchaeota* abundance is <10% of total cells near the surface, increasing to 39% of total cells below 1,000 m, which is equal to the proportion of the bacterial population there. *Euryarchaeota* never comprise more than a few percent of the population at any depth (270). Similarly, the proportion of *Crenarchaeota* in the North Atlantic increases with depth (from 18.5% of cells at the surface to 26.4% of cells at 2,700 m), while the proportion of *Euryarchaeota* remains somewhat constant with depth (214). Below 100 m in the North Atlantic, archaeal cells are more abundant than bacterial cells. In contrast, bacterial cells are always more abundant than archaeal cells in the water column in the Antarctic (214), suggesting a latitudinal variance in the relationship of archaea to bacteria. The diversity of archaea has been observed to increase with depth, due in part to the increasing proportions of diverse ammonium-oxidizing *Crenarchaeota* (70).

Typical deep ocean bacterial communities contain *Alphapro-*

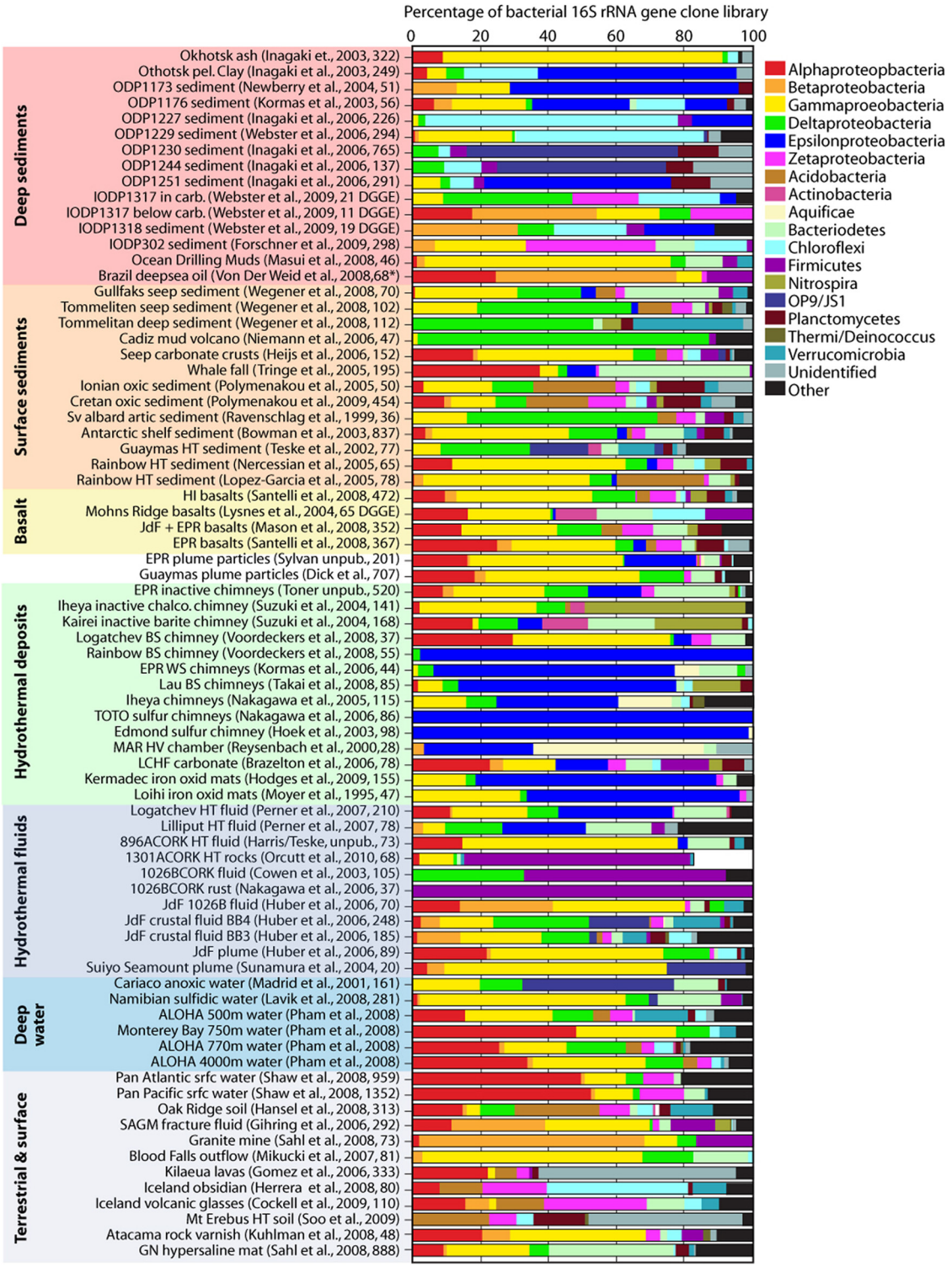

FIG. 7. Composition of bacterial communities in various dark ocean habitats based on the percentages of different bacterial phyla documented in clone libraries of the 16S rRNA gene containing nearly full-length sequences. Each line represents a different sample set from one environment. The numbers after the references indicate the numbers of sequences in the sample clone library. Samples are grouped by habitat type, as indicated in the far left margin. Abbreviations and acronyms: pel., pelagic; ODP, Ocean Drilling Program; IODP, Integrated Ocean Drilling Program; carb., carbonate; HT, hydrothermal; HI, Hawaii; JdF, Juan de Fuca Ridge; EPR, East Pacific Rise; chalco., chalcopyrite; BS, black smoker; WS, white smoker; HV, hydrothermal vent; LCHF, Lost City hydrothermal field; ALOHA, Station ALOHA, Pacific Ocean, north of Hawaii; srfc, surface; SAGM, South African gold mine; GN, Guerrero Negro, Baja, CA. References correspond to reference numbers 60, 64, 88a, 93, 124, 166, 186, 196a, 202, 213, 215, 219a, 224, 232, 240, 242, 289, 290, 293, 307a, 335a, 346, 346a, 354, 357a, 372, 386, 392, 397, 400, 407, 409, 414, 424, 442, 443, 444, 447, 449, 456, 467, 476, 479, 499a, 506a, 518, 520, 535, 544, 566, 580a, 581, 590, 591, and 592.

teobacteria (*Rickettsiales* and *Rhodospirilliaceae*), *Deltaproteobacteria* (the SAR324 clade and *Nitrospina*), various members of the *Gammaproteobacteria*, *Chloroflexi* (the SAR202 clade), *Actinobacteria*, *Deferribacteriales*, and the SAR406 and Agg47 clades, which have no cultivated members (Fig. 7) (70, 113, 444). In comparing different water depths and locations, roughly one-third to one-half of detectable prokaryotic phylotypes are ubiquitous in the aphotic pelagic ocean (216, 623). For example, the SAR202 clade of uncultivated *Chloroflexi* is observed to comprise roughly 10% of the microbial community below the euphotic zone at multiple locations, including the oligotrophic open-ocean Atlantic water column (at the Bermuda Atlantic Time Series [BATS] station), the oligotrophic open-ocean Pacific water column (at the Hawaiian Ocean Time Series [HOTS] station), and the Oregon coast (382), although the physiology of this group is currently unknown. Commonly occurring gammaproteobacteria in the aphotic pelagic ocean include *Colwellia*, *Shewanella*, *Alteromonas*, and *Pseudoalteromonas* relatives, based on 16S rRNA gene clone libraries (336, 623). Massively parallel sequencing of aphotic waters from the North Atlantic revealed *Alpha-* and *Gammaproteobacteria* to comprise the most abundant groups (505). Additionally, one of the largest and most detailed marine gene sequencing efforts to date also observed stratification patterns in numerous groups of prokaryotes (113). In that study, 16S rRNA gene sequences from below 200 m were clearly different from those from the euphotic zone, and sequences from 500 m and 770 m were more similar to each other than to those at 4,000 m. A follow-up survey revealed a number of uncultivated proteobacterial clades specific to the dark water column (444), further highlighting the lack of cultivated representatives from dark ocean habitats hampering efforts to understand the functioning in these environments. Metagenomic analyses of an uncultivated clade of bacteria—the SUP05 group (584), commonly found in oxygen-deficient waters (624)—indicated that this group may be involved in autotrophic sulfur oxidation, providing a genome-based foundation for understanding the roles that these microorganisms may play in a fluctuating water column habitat.

As observed for prokaryotic microbial communities, microbial eukaryotic communities also display vertical stratification patterns in the aphotic ocean (92, 337, 421). Interestingly, sequencing efforts have revealed that the majority of detected microbial eukaryotic taxa in the dark ocean occur with low frequency, with only a few dominant groups (92), although the mechanism underlying this large number of rare species is currently unexplained. Of the dominant groups, members of the *Eukarya* kingdoms Rhizaria (Radiolaria), Chromalveolata (Acantharia, Alveolata, and Polycystinea), and Fungi are common (70, 92, 421). Studies on the distribution of fungi in the world's oceans suggest that a number of dominant species are found only in deep-water samples (37). Furthermore, a recent examination of protistan diversity in the Sargasso Sea and the Gulf Stream found the *Eukarya* groups Acantharea, Polycystinea, and Euglenozoa to be unique to 2,500 m versus the euphotic zone (92). In that study, only 9% of protistan species were detected in both the shallow- and deep-water samples, and more than 30% of species were unique to the deep-water samples. Massively parallel sequencing of the V9 region of the 18S rRNA gene from deep-water samples from near Hawaii also indicated that ~30% of deep-water species were not found in surface waters (70). In the same study, eukaryal diversity was found to decrease with depth, although the level of diversity within the *Eukarya* was nearly as high as that for *Bacteria* and ~20 times higher than that for *Archaea*.

Similarly to their hosts, deep-water viruses also exhibit vertical stratification patterns. Viral abundance in the water column increases more substantially with depth than is the case for prokaryotes, yielding higher virus-to-prokaryote ratios in the deep ocean than at the surface (199, 207, 431). Considering that there are lower heterotrophic production rates in the deep ocean, the higher virus-to-host ratio suggests higher rates of lysogenic than lytic infection in the dark ocean. Viral diversity appears to be relatively low in the deep ocean (431), although studies on this subject are limited.

Diversity surveys throughout the water column have revealed an increase in microbial diversity in the deepest waters of the ocean (37, 216, 444). For example, a study using the fingerprinting technique ARISA documented higher microbial diversity in the deepest samples from a global data set taken at 3,000 m (216). Another study found that microbial yeasts were more diverse in the deepest samples from multiple stations (37). Archaeal diversity also increased with depth, as discussed above (70). However, as has been observed in other marine environments (233), archaeal diversity in the aphotic pelagic ocean consistently appears to be lower than that of bacteria (357). The factors explaining increases in diversity in the deepest portions of the ocean are unclear. Some have suggested that deep-water circulation is a potential factor contributing to diversity trends (113, 444). For example, dispersal of piezophiles by deep-sea currents is one hypothesis suggested to explain their biogeography (307).

Directed studies exploring the relationship of prokaryotic microbial diversity to deep-water circulation and benthic processes are needed. Such studies should quantify the vertical and lateral extents into the water column where benthic processes can influence diversity or microbial activity in general. As one example, it is known that the concentrations of dissolved metals are higher in water masses above hydrothermal vents, with elevated concentrations existing for several tens of kilometers away from the source vent (e.g., see reference 96), indicating that benthic processes and water circulation could influence deep-water peaks in microbial activity. Similarly, benthic processes in other dark ocean habitats may all have far-field influences in the pelagic ocean, depending on the physics of water mass movement.

Marine Sediments

Marine sediments have been studied extensively in the past decades, and the available knowledge provides a coherent framework for understanding the rates of microbial activity and composition of microbial communities in marine sediments. However, there are still many open questions, and new discoveries are continually being made. Our understanding of sedimentary processes is strongly skewed toward shallow depths (<1 m) (Fig. 3A and 6D and F) and continental margins, where the majority of samples have been obtained; data sets from open ocean sites and deep sediment (Fig. 3D) are scarce.

General physical and chemical characteristics. Marine sediments result from the accumulation of particles that sink to the seafloor from the overlying water column. A layer of sediment blankets the majority of the seafloor, although the thickness of this layer varies from relatively thin to absent near newly formed crust at midocean ridges and beneath low-productivity zones of the ocean gyres to deposits on the order of 10 km thick at trenches and some highly productive continental margins (Fig. 1). The composition of the particulate matter in sediments depends on the origin of the deposited material, ranging from biological-based oozes of coccolith or diatom shells that "rain" down from the surface ocean to eroded terrestrial matter delivered by rivers, accumulations of hydrothermal vent-derived minerals, and deposition of wind-blown particles from land. The local importance and abundance of these different sediment particle origins vary based on proximity to the sources. Sediment particle size can vary over many orders of magnitude, from small-scale clay particles to macroscopic sands and pebbles, which has a significant impact on the porosity of sediments and ultimately affects fluid and chemical transport in sediments. The transport of chemical compounds in sediment is driven mainly by molecular diffusion against chemical gradients and thereby limits the supply of substrates to microorganisms. Advective transport of substrates occurs only where fluid is actively moved—these sites are often studied intensively as hot spots of microbial activity due to the higher availability of substrates. Other mixing processes, such as bioirrigation and bioturbation by sediment-dwelling macrofauna, can also occur and affect substrate availability.

Marine sediments are generally classified according to their proximity to a tectonic plate or land boundary. The most well-studied environment is the continental margin, which represents about 20% of the aerial coverage of marine sediments (Fig. 1; Table 1). Passive continental margins are those that do not coincide with a tectonic plate margin, for example, the Gulf of Mexico and the Atlantic Ocean coasts. Within the passive continental margin, the sedimentary environment can be subdivided into the continental shelf (less than ~200 m in water depth) and the continental slope (~200 m to 1,000 to 2,000 m). In contrast, active continental margins are those that do coincide with a tectonic plate margin, for example, all along the Pacific Ocean, where plate subduction occurs in a variety of settings. Subduction leads to both the burial and dehydration of sediments on the subducting plate and to accretion of sediments on the overriding plate. The seafloor underlying the open ocean is referred to as the abyssal plain and occurs at an average water depth of 4 km and with significantly less organic matter delivery than at the continental margins.

Vertically, sediments are classified as either shallow/surficial or deep/subsurface. Biogeochemically, shallow sediments represent the horizon from a few centimeters to meters of depth, where the most pronounced gradients in geochemical parameters occur in tandem with higher local rates of microbial activity. Below this horizon, lower densities of cells, lower turnover rates, and more stable geochemical gradients pervade (119, 409, 433, 436, 595). This shallow-deep sediment classification is sometimes also defined operationally, driven by how sediments are collected for research (i.e., by oceanic drilling [Fig. 6E] or other coring technologies [Fig. 6D]). This operational threshold depth has varied in the literature between 10 cm and 10 m (121, 608).

Sedimentary microorganisms depend on substrates that are either deposited with the sediment or diffuse into it from the overlying seawater or underlying crust. The quantitatively most important substrate is organic matter that is deposited, degraded, and "recycled" or remineralized by oxidation back to carbon dioxide. Rates of remineralization depend on both the quantity and quality (e.g., freshness, terrestrial versus marine origin, etc.) of organic matter (211, 308, 331, 418). In addition, organic matter remineralization rates depend on the availability of terminal electron acceptors, which, as noted in the section Dark Ocean Habitats—an Overview, are utilized in a sequential order based on the energetically most favorable reactions occurring first. Thus, there is a typical pattern of redox processes occurring with depth in the sediment—the diagenetic sequence—from respiration with oxygen to nitrate reduction, metal oxide reduction, sulfate reduction, and finally methanogenesis (Fig. 2). Increasingly, the delivery of oxidants from below, at the contact between the oceanic crust and lower sediments, is gaining recognition as a source of metabolic fuel to otherwise substrate-depleted deep sedimentary environments (Fig. 2) (119, 146). Seawater and hydrothermal fluids circulate through oceanic crust underneath sedimentary deposits at a distance away from midocean ridges. Depending on the degree of reaction of the fluids within the crust, they may contain oxygen, nitrate, and sulfate, which can diffuse upward into the sediments from the crust, providing additional electron acceptors to deeply buried marine sediments. The quantitative significance of microbial processes based on these sources is unknown at present.

Metabolic reactions. The highest rates of photosynthesis, the source of most sediment organic matter, take place in the water column over the shallow and nutrient-rich continental shelf; therefore, the input of organic matter to sediments decreases with increasing distance from land. The rates of microbial organic matter remineralization decrease accordingly with increasing water depth and distance from land, with the highest rates in shelf sediments (Table 3). Intriguingly, rates of metabolic activity do not appear to show a trend with latitude—rates in permanently cold sediments of the high Arctic are similar to those measured in more tropical locales, suggesting temperature-adapted microbial communities (21).

In highly active sediments, the underlying factor that determines the rates of organic carbon remineralization is the availability of terminal electron acceptors, as these compounds are consumed more rapidly than they can be supplied. This can result in the burial of a larger percentage of deposited organic matter in sediments underlying organic-rich regions as the organic matter escapes oxidation. In shelf sediments, the sequence of microbial diagenetic processes generally occurs in well-defined zones (Fig. 2). The top sediment layers (mm to cm) are characterized by a rapid depletion of oxygen followed by nitrate reduction and manganese and iron reduction. The quantitatively most important organic matter remineralization pathway is sulfate reduction (Table 3), which can account for 25 to 50% of the organic matter oxidation in shelf sediments (78, 86, 256, 459). The relative contributions of the other electron acceptors are determined mainly by overall organic carbon oxi-

TABLE 3. Sulfate reduction rates and organic carbon (C_{org}) content in surface sediments from various locations, grouped according to water depth

Location	Water depth (m)	C_{org} (wt %)	Sulfate reduction rate (mmol m^{-2} day^{-1})	Reference
Inner shelf	0–50			
Baltic Sea, Kysing Fjord	0.5	5–12	4–88	246
Great Barrier Reef	5	1–2	3–21	596
Baltic Sea, Norsminde Fjord	1	6–9	60	205
Baltic Sea, Eckernförde Bay	28	4–5	4–11	562
Baltic Sea, Aarhus Bay	15	3.3	3	H. Fossing et al., unpublished data
North Sea, Kattegat	14–25	1–13	1–7	257
Pacific Ocean, Chilean coast	29–37	6–9	0–56	155, 546
Lower shelf	50–200			
North Sea, Kattegat	65–200	7–12	<3	257
Black Sea (anoxic)	62–130	2–3	<2	589
Pacific Ocean, Mexican coast	97–190	3–6	<3	208
Pacific Ocean, Washington coast	85–137	1–2	<8	208
Pacific Ocean, Chilean upwelling zone	57–122	5–6	<33	155, 546
Atlantic Ocean, Gulf of St. Lawrence	130–355		<1	128
Slope	200–1,000			
Baltic Sea, Skagerrak	380–695	10	<3	79
Black Sea (anoxic)	181–396	6–8	<2	260, 589
Atlantic Ocean, Gulf of St. Lawrence	355	12	4	128
South China Sea	350–945	0–1	<5	322
Pacific Ocean, Chilean upwelling zone	307–891	2–5	<1	418
Pacific Ocean, Mexican coast	220–1,020	7–12	<2	208
Pacific Ocean, Washington coast	219–1,025	2–3	<3	208
Atlantic Ocean, Namibian upwelling	855	3–7	1	154
Abyss	>1,000			
Pacific Ocean, Mexican coast	1,500–3,065	4–6	<0.2	208
Pacific Ocean, Washington coast	1,994–2,746	1–2	<0.2	208
Sea of Japan, Ulleung Basin	1,570–2,143	2–3	<2	309
Black Sea (anoxic)	1,176–2,045	5	<0.4	311, 589
South China Sea	1,000–1,620	0–1	<0.3	322
Atlantic Ocean, Namibian upwelling	1,311–4,766	1–7	<2	154
Pacific Ocean, Chilean upwelling zone	1,007–2,744	2–4	<3	155, 418, 546, 562

dation rates and are limited by their availability; thus, nitrate reduction or metal reduction can be more dominant at river mouths with agricultural input or in areas with high deposition of terrestrial dust. Because of the high rates of organic matter degradation to carbon dioxide/bicarbonate and the rapid depletion of other available terminal electron acceptors, continental shelf sediments are dominant areas of microbial methanogenesis from bicarbonate and hydrogen, leading to the accumulation of significant amounts of methane (164). It is estimated that about 10% of total organic matter is converted into methane by methanogenesis (88). The methane diffuses upwards to the sulfate zone, where it is oxidized anaerobically in concert with sulfate reduction (Table 4), forming the SMTZ, with opposite methane and sulfate concentration profiles, a hallmark of diffusive organic matter-rich shelf sediments.

In the transition zone between continental shelf and the open ocean, metabolic activity varies depending on the setting, the depositional conditions, and the organic matter supply. For example, rates of microbial activity in sediments underlying oceanic upwelling zones are higher than those in comparable sediments on continental margins. In upwelling areas, where deep ocean water rises to the surface due to physical forcing, the nutrients delivered stimulate growth of plankton in the surface ocean, and hence result in high productivity in the sediments that receive a large input of organic matter. For example, on the Chilean margin, sulfate reduction rates are much higher than at nonupwelling sites (546). In contrast, sediments that receive very little organic matter have quantitatively lower sulfate reduction rates because organic matter is oxidized through other pathways

TABLE 4. Rates of sulfate reduction fueled by methane in surficial marine sediments (based on rates of anaerobic oxidation of methane [AOM] in the sulfate-methane transition zone)[a]

Location	Habitat	Water depth (m)	Sulfate reduction rate by AOM (mmol m^{-2} day^{-1})	Reference
Inner shelf	Diffusive	0–50	0.05–17.04	246, 548
Lower shelf	Diffusive	50–200	0.31–1.14	286, 458
Slope	Diffusive	200–1,000	0.03–0.47	563
	Cold seep	200–1,000	0.056–99	264, 561
Abyss	Mud volcano	>1,000	0.06–1.58	414, 417

[a] The data listed are the highest and lowest values in the literature.

before it reaches the sulfate zone.

The open ocean beyond the continental margins is characterized by low primary production in surface waters, leading to very little input of organic matter to the sediments below. Correspondingly, microbial cell densities and rates of carbon remineralization are also low, on average, in sediments underlying the open ocean (Table 3) (e.g., see references 86, 119, 121, and 256). Due to the low carbon remineralization rates in the abyssal plains, oxygen is consumed slowly and can diffuse centimeters to meters into sediments (121, 462). Therefore, open ocean sediments are generally characterized by relatively high oxygen content, with oxygen thus being the dominant terminal electron acceptor for remineralization. The concentrations of other electron acceptors, such as nitrate and sulfate, show little variance with depth.

In addition to organic matter remineralization, the availability of methane influences microbial distribution and activity. On the continental shelf and slope, where gases (mainly methane) migrate toward the sediment surface as either gas bubbles or methane-rich fluids, unique habitats such as gas seeps and mud volcanoes are formed (Fig. 4). Gas seeps can occur when the accumulation of methane results in pressure build-up, which leads to fracturing in the sediment and subsequent gas release. Gas seepage can also be related to the buoyant movement of buried brines and the compaction and expulsion of fluidized mud from high deposition rates and tectonic forcing (229, 265, 274, 284). Methane in these environments can originate from a geological source (i.e., production from the thermogenic breakdown of buried organic matter) or from biological sources (i.e., methanogenesis) (481, 482). Seeps are frequently found on the eroded slopes of organic-rich continental margins, such as in the Black Sea (368, 401, 449), or in areas with gas hydrates (e.g., see references 296 and 373). The high methane fluxes at gas seeps fuel distinct chemosynthetically based ecosystems of microorganisms and macrofauna (Fig. 4) (284). Rates of microbial methane oxidation in seep sediments are very high compared to those in nonseep sediments at appreciable depths, and the zone of activity occurs much closer to the sediment surface than the SMTZ in diffusion-dominated systems (264, 561). This process often leads to the formation of carbonate deposits, for example, the spectacular carbonate and microbial mat chimneys formed in the anoxic water of the Black Sea (368) (Fig. 4A). The high microbial methane oxidation rates at gas seeps do not completely prevent the escape of methane to the water column, but nonetheless these processes present an important barrier (sometimes referred to as the microbial methane filter) against the release of sedimentary methane into the atmosphere, which has important implications for the budget of this greenhouse gas (218, 460).

Similar to gas seeps representing conspicuous oases of sedimentary activity, dense organic matter deposits at the seafloor, such as animal carcasses (Fig. 4B) and wood falls, also support microbial and faunal communities that are more active than those in surrounding sediments (29, 194, 503, 504, 564). Where such "food falls" occur, a succession of microbial and macrofaunal communities colonize the deposit, and high levels of biological activity result (194). The influx of more labile organic carbon to the sediment leads to an increase in sediment microbial activity and the development of shallow sulfate reduction zones in the sediment (564).

In highly active sediments, the sulfide produced by sulfate reduction diffuses upwards and is oxidized by other electron acceptors, or it diffuses downwards, where it can precipitate as iron sulfide and elemental sulfur. Although it removes only a relatively small fraction of the reduced sulfide, pyridization is important as the only sink for electrons that are not eventually reoxidized and accounted for in the oxygen uptake of the sediment (192, 193). In sediment with high sulfate reduction rates, some of the sulfide produced by sulfate reduction reaches oxygen-bearing portions of the sediment, where it can be utilized by chemolithotrophic sulfide oxidizers. Conspicuous examples of microorganisms that mediate this process are *Beggiatoa* bacteria, which form multicellular mobile filaments of up to 1 cm in length and have been observed as mats on sediments with very steep oxygen gradients (Fig. 3B and 4D) (258). These bacteria are capable of gliding, by expulsion of mucous sheaths, toward favorable substrate gradients. *Beggiatoa* organisms are able to store granules of elemental sulfur inside the cells as an intermediate of sulfide oxidation. Similar sulfur granules are also found in two other giant sulfide-oxidizing bacteria—*Thioploca* and *Thiomargarita*—who use nitrate as an electron acceptor instead of oxygen (261, 496) and are thus important in linking the sulfur and nitrogen cycles. *Thioploca* vacuoles have been observed to contain nitrate concentrations 20,000 times higher than those in ambient seawater (167), allowing these microorganisms to overcome the challenge of sulfide and nitrate not coexisting in the same zones. Filaments of cells arranged in a vertical sheath of up to 7 cm in length commute between the sediment surface, where they take up nitrate from seawater, and the zone of sulfate reduction, where they acquire sulfide. Hence, these microorganisms can shuttle nitrate to sediment depths where it is depleted, overcoming the typical sequence of diagenetic reactions. The largest bacteria discovered so far, *Thiomargarita* spp., do not seem to use mobility to accumulate nitrogen but rather are able to survive long intervals by using the nitrate stored in their vacuoles before sediment resuspension events allow them to take up nitrate again (496). Their metabolism during these intervals is also supplemented by energy generation from the degradation of polyphosphate, which is also stored as polyphosphate deposits in the cells (497).

The breakdown of organic matter not only provides a carbon source to sedimentary microorganisms but is also the main source of organic nitrogen compounds, mainly amino acids. Along with the recently discovered possibility of direct nitrogen fixation by sedimentary microorganisms (44, 107), these are the main inputs of nitrogen to the sediments. Remineralization of organic nitrogen leads to the accumulation of ammonium in sediments. In oxic sediments, ammonium is oxidized to nitrite and nitrate in the process of nitrification. This nitrate is then either used in the microbial process of denitrification, whereby the nitrate is converted stepwise to nitrite and N_2 by two different groups of microorganisms, or consumed in the pathway of dissimilatory nitrate reduction to ammonium (DNRA) (271, 507). In anoxic sediments, ammonium is also oxidized microbially to N_2 by anammox (547), although global budgets of nitrogen cycling pathways in continental margin sediments are continually being revised. N_2 production rates are higher in regions where overlying primary

TABLE 5. Sulfate reduction activities in deep marine sediments, calculated from flux of sulfate into sediments from the seafloor based on SO_4^{2-} profiles[a]

Location	ODP (IODP) leg (site)	Depth (m)	SO_4^{2-} flux (mmol cm^{-2})	Reference
Blake Ridge	164 (994)	210–687	5×10^{-5}	ODP database
	164 (994)	350–624	1×10^{-4}	ODP database
Japan Sea	181 (1119)	300–472	2×10^{-4}	ODP database
	181 (1122)	122–590	1×10^{-3}	ODP database
Nankai Trough	190 (1773)	140–440	1×10^{-3}	ODP database
Cascadia Margin	204 (1244)	128–297	7×10^{-4}	ODP database
	204 (1251)	255–440	5×10^{-4}	ODP database
Peru	201 (1226)	265–420	1×10^{-3}	ODP database
	201 (1229)	83–128	8×10^{-3}	ODP database
Juan de Fuca Ridge	168 (1023)	145–200	1×10^{-2}	138
	168 (1026)	125–190	1×10^{-2}	138
	(301 [1301])	120–250	1×10^{-3}	146

[a] For flux values not reported in the literature, sulfate concentration data were obtained from publicly available databases operated by the ODP.

productivity is higher (565). On continental shelves, N_2 production is fueled mainly by denitrification, while on the slope the relative importance of anammox increases (565). It is estimated that 20 to 40% of the nitrogen loss (i.e., N_2 production) from sediments can be attributed to the anammox process (e.g., see references 147, 172, and 547). For further details about the intricacies of the nitrogen cycle, readers are referred to recent reviews on the subject (172, 464).

In continental margin sediments, benthic macrofauna, such as Polychaeta, Echinodermata, Arthropoda, Annelida, bivalve molluscs, and Hemichordates, can have significant impacts on the sediment biogeochemistry by bioturbation (i.e., mixing of sediment particles) and bioirrigation (i.e., flushing seawater through burrows). The distribution and abundance of benthic macrofauna vary with water depth, latitude, organic carbon content, and oxygen concentrations (316). Dissolved oxygen affects mainly species richness, whereas organic matter content determines species dominance (316). The activity of benthic macrofauna facilitates the transport of bottom seawater to sediment layers beyond which oxygen can penetrate by diffusion, thereby enhancing the sediment-water solute exchange (e.g., see references 9, 328, 365, and 369). The bioturbation zone of organic matter-rich sediments undergoes oscillation between oxic and anoxic conditions (292), which strongly influences organic carbon remineralization. Bioirrigation stimulates oxygen consumption, nitrification, and nitrate reduction (413), and thus the capacity for organic matter remineralization (291). Bioturbation promotes the burial of settled organic matter to deeper sediment layers (315), which can result in stimulation of older organic matter degradation (10, 77, 291). Bioturbation and bioirrigation also increase the supply of nutrients, especially ammonium, to the overlying nitrogen-limited waters, thereby influencing primary productivity (328, 413).

Moving deeper into the sediments, general knowledge about microbial activity in deeply buried sediments (Fig. 3D) has grown considerably in the last 2 decades. The study of deeply buried life has been hampered by sample access, which often requires specialized oceanic drilling vessels (Fig. 6E). Early examination of sediments demonstrated that microbial life persisted as deeply as early long-core technologies were able to sample (381, 626). Geochemical profiles on deep cores further revealed gradients that could only be explained by microbial activity (87, 428, 606). These observations prompted a flurry of sampling efforts and studies on deeply buried life since the late 1980s (47, 102, 119, 146, 219, 240, 323, 433, 488) and have led to suggestions that, given the large volume of deep sediments, vast numbers of microorganisms may be buried and active in this habitat. Trends of cell density with sediment depth have been used to estimate that marine subsurface sediments might contain as much as 1/10 (434) to 1/3 (608) of the biomass on Earth, although local cell density is relatively low compared to that in surficial environments.

Deeply buried sediment microorganisms must survive under extremes of energy and growth limitation; it is not currently clear how the organisms in this "deep biosphere" are sustained (259). Understanding the mechanisms controlling geochemical gradients and the corresponding metabolic activities and community patterns in deep marine sediments is an active area of investigation. The availability of electron donors in deep sediments is not well understood, although at first order, the size of deep sediment microbial communities correlates positively with organic carbon contents of the host sediments, consistent with patterns observed in other habitats (i.e., higher cell densities in sediments that are more organic rich [120, 323, 436]). This observation leads to the hypothesis that organic matter derived from the upper ocean that has escaped remineralization in shallow sediments fuels the deep sedimentary biosphere. The degradation of organic matter in sediments decreases logarithmically with depth (370), and thus organic matter is depleted to low levels below the zone of sulfate reduction. The remaining pool of organic matter is considered refractory and not bioavailable (77, 370). However, some evidence suggests that buried inert organic matter becomes bioavailable again in deep sediments due to the increasing temperature with depth allowing modification of the organic matter, which can then be utilized by microorganisms in the deeper sediment layers (595). In addition to organic carbon, other electron sources might play a significant role in life in deep subsurface environments. For example, increased cell numbers based on oxidation of methane are also found at deep sulfate-methane transition zones (47, 119). Also, recent surveys of functional genes and stable carbon isotopes of acetate in deep sediment pore water indicate that acetogenesis from organic matter and/or hydrogen and carbon dioxide is another important metabolic pathway fueling the deep sedimentary biosphere (313). Although rates of activity in deeply buried

sediments may be low on the local scale (Table 5) due to low organic content compared to that in surficial sediments, considering the size of the sediment reservoir (Table 1), the summed rate of activity is quite large and could significantly impact some global biogeochemical cycles. For example, D'Hondt et al. (120) calculated that the total annual subsurface respiration at open ocean sites is roughly 10^{-8} mol CO_2 cm^{-2}, which is several orders of magnitude lower than that at continental margin sites (10^{-6} to 10^{-5} mol CO_2 cm^{-2}), but considering the vast area of open ocean sediments compared to the continental margin, these rates are still significant.

At the interface between sediment and the underlying ocean crust, the relatively oxidized fluids circulating through oceanic crust provide oxidants to electron acceptor-poor deep sediments (119, 146), possibly stimulating microbial activity. For example, sediment geochemical profiles from the eastern flank of the Juan de Fuca Ridge indicate that sulfate from crustal fluids diffuses upwards, fueling sulfate reduction and methane oxidation at depth within the sediment (138, 146). Similarly, at the Peru Margin, elevated sulfate derives from crustal fluids and diffuses upwards into the overlying deep sediments (119, 436). Estimates of the sulfate flux into sediment indicate that a low but substantial rate of sulfate consumption could be fueled by this basement oxidant source.

Other redox reactions may also fuel microbial activity in deeply buried sediments, although documentation of their occurrence or importance is scarce. For instance, sulfide oxidation via Mn reduction has been proposed as a potential metabolic activity (57). Furthermore, radiolysis of water to form hydrogen, fueled by the natural radioactivity of uranium and other elements buried in sediments, has been proposed as a mechanism to generate electron donors in deep marine sediments, especially in organic matter-poor sediments (52, 121, 321, 381), although this pathway has not been verified. Dehalogenation reactions, where halogenated organics are reduced with hydrogen or another electron donor to provide metabolic energy, may also support microbial activity in deep marine sediments (180), but this pathway also requires validation.

Microbial distribution and diversity. The accumulation of data on prokaryotic cell densities in marine sediments over the past few decades has revealed several patterns in microbial distribution. The abundance of cells in sediments decreases logarithmically with increasing sediment depth, tracking the decrease in organic carbon concentration and remineralization rates. Cell densities in sediments with moderate to heavy organic carbon loading range from an average of 10^8 to 10^9 cells cm^{-3} in surface sediments to 10^6 to 10^7 cells cm^{-3} in sediments hundreds of meters deep (434). Cell abundances are also higher in sediments closer to shore (with higher organic carbon content) than in sediments underlying the open ocean (120). Recent work with sediments underlying the South Pacific Gyre, where the lowest biomass production in the ocean occurs in the overlying surface waters, found that cell abundances were 10^3 to 10^4 cells cm^{-3} in the first 10 m below the seafloor (121). Overall, cell densities in deep marine sediment also tend to track the content of sedimentary organic carbon (42, 101, 323), and they have even been shown to track paleoclimatic productivity patterns (3).

Early work that documented the presence of microbial life deep within sedimentary sequences raised questions about the sources of energy that support microbial life and the metabolic state of this biosphere. Evidence now suggests that a significant fraction of observed cells in deep sediments are alive, based on the presence of rRNA (488). However, it appears that deep sediments host more live cells than can be explained by the available energy-generating pathways from geochemical gradients and the low observed metabolic rates. The same calculations imply that deep sedimentary microbes would have regeneration times on the order of thousands of years (259), which is far beyond the limits currently known in biology. Trying to find solutions to these apparent paradoxes is an active area of investigation. Another unresolved characteristic of deep sediment microbial ecology regards the relative proportions of archaea and bacteria. In deep sediments, there are conflicting results about which domain of life predominates. For example, some groups have found the *Archaea* to be the dominant members of deep sediment microbial communities (47, 323), while others provide evidence that the *Bacteria* are more abundant (240, 488). Some explanation for this discrepancy may be due to methodological biases, as the low cell densities of deep sediments push methods to their limits of detection.

Sediment microbial community composition varies according to oxygen content, hydrothermal influence, carbon content, and sediment depth, with little relationship to latitudinal or ocean basin variation (Fig. 7 and 8). Oxic surficial sediments harbor relatively diverse bacterial communities in terms of numbers of phyla based on 16S rRNA gene clone libraries, with notable representation from the *Alpha-*, *Delta-*, and *Gammaproteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Planctomycetes* (60, 447, 448). In contrast, archaeal diversity in oxic surficial sediments is relatively low, with dominant representation from the marine group I (MGI) *Crenarchaeota* (60, 195). Surficial sediments with high rates of activity and more reducing conditions, such as from gas seeps or near animal carcass falls, host strikingly different microbial communities (195, 213, 415, 566, 592). At gas seeps, which are characterized by high sulfate reduction and methane cycling, the bacterial communities are dominated by *Deltaproteobacteria* (which contain the majority of known sulfate reducers), and archaeal communities are dominated by members of the *Methanomicrobia*, namely, methanogens and anaerobic methane-oxidizing archaea. Organic-rich deep sediments support bacterial communities dominated by the uncultivated OP9/JS1 phylum, whereas more organic-poor sediments host bacteria related to *Chloroflexi* and *Proteobacteria* (240, 242, 289, 409, 591). Many archaeal communities in organic-rich deep sediment are dominated by the uncultivated deep-sea archaeal group/marine benthic group B (DSAG/MBGB) clade as well as the uncultivated miscellaneous crenarchaeotal group (MCG) and MGI (240, 242, 289, 409, 436, 508, 509). Thermally influenced surficial and deep sediments exhibit similar characteristics, with increased representation from groups more typically found in hydrothermal environments, such as the *Epsilonproteobacteria* and the *Archaeoglobus*/*Thermococcus*/*Methanococcus* (A/T/M) groups (407, 422, 544).

A surprising discovery in the last few years has been the finding of thermophilic bacteria in permanently cold surficial sediments of the Arctic (235, 236). When the cold sediments were heated, a population of thermophilic spore-forming *Desulfotomaculum* species was induced that mediated sulfate reduction. The closest relatives of the induced *Firmic-*

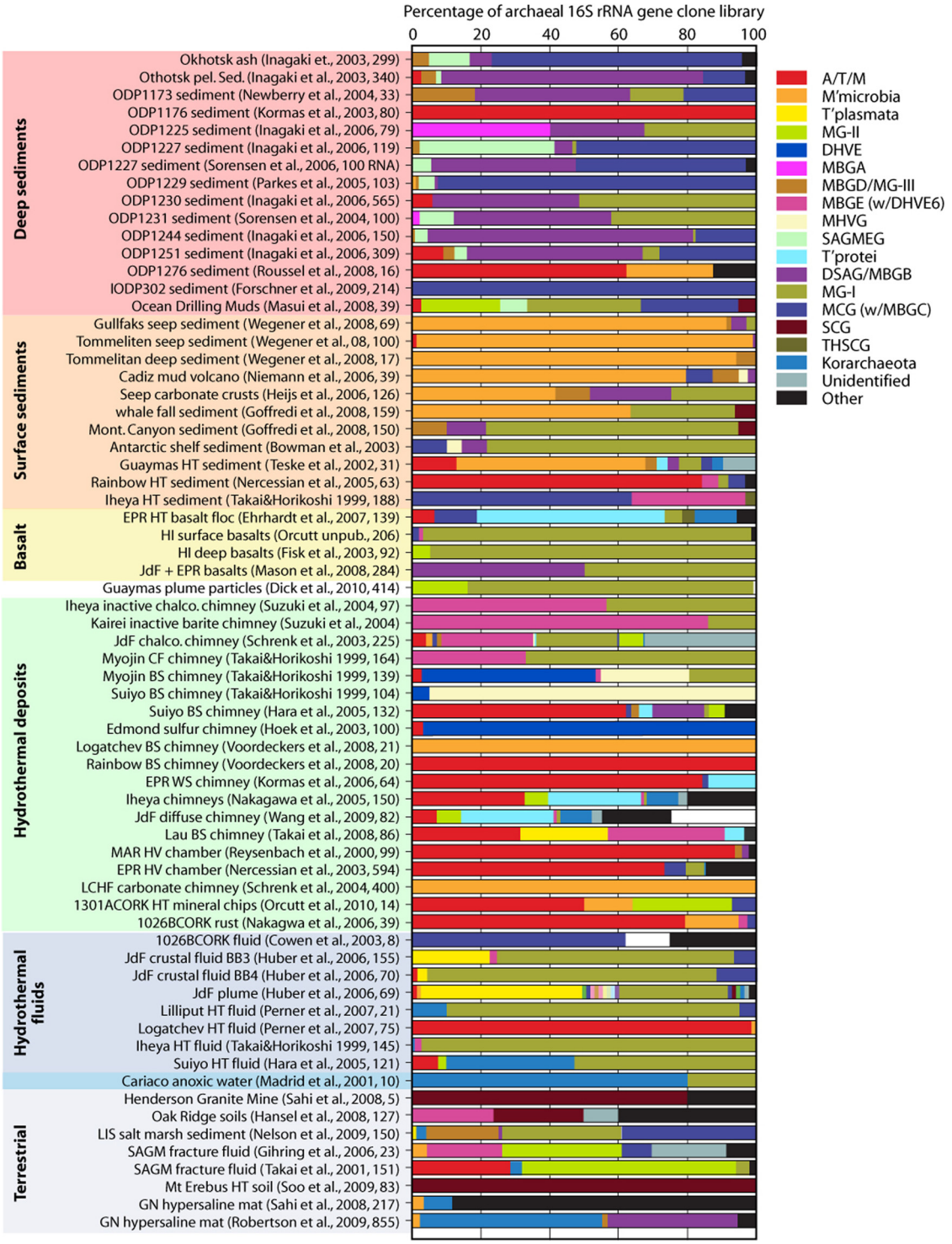

FIG. 8. Composition of archaeal communities in various dark ocean habitats based on the percentages of different archaeal phyla documented in clone libraries of the 16S rRNA gene containing nearly full-length sequences. Each line represents a different sample set from one environment. Samples are grouped by habitat type, as indicated in the far left margin. Abbreviations and acronyms are the same as in Fig. 7, with the addition of LIS for Long Island Sound. See reference 545 for more information on archaeal group naming conventions. References correspond to reference numbers 60, 93, 124, 135, 163, 166, 186, 195, 202, 206, 213, 224, 232, 240, 242, 289, 290, 346a, 354, 392, 397, 406a, 407, 408, 409, 417, 424, 436, 442, 443, 467, 469, 473, 475, 476, 493, 494, 506a, 509, 520, 524, 535, 544, 581, 585, and 592.

utes originate from warm subsurface petroleum reservoirs and hydrothermally influenced oceanic crust, indicating a hydrothermal origin of the sediment spores and suggesting a dispersion of hydrothermal-origin spore formers to geographically distant cold environments. A high dispersion rate for resident sediment microorganisms is also indicated by the comparison

of oxic sediment communities along the flow path of deep ocean currents (484), suggesting a nearly global connectivity of Earth's dark ocean habitats for microbial distribution.

Less is known about eukaryotic microbial ecology in marine sediments, although it is important to understand sediment eukaryotes because of their impact on the breakdown of organic carbon. Initial studies of near-surface hydrothermal sediments indicated that many sediment eukaryotes cluster into deeply branching groups within the eukaryotic lineage (129, 333, 485). Furthermore, the phylogeny of sediment eukaryotes suggests that they may be adapted for life in microaerophilic and/or anaerobic sediments. Eukaryotic DNA was not recovered below 3 cm of depth in hydrothermal sediments from the Guaymas Basin, although bacteria and archaea were detected at >20 cm of depth in the same sediments, indicating that eukaryotes may have a smaller range of habitability than prokaryotes (129). At another hydrothermal site, sediment eukaryotic communities were more diverse than communities hosted in the hydrothermal fluids or seawater, with the sedimentary eukaryotic community containing unique Alveolates and Kinetoplastids, flagellated protists that are commonly parasitic (333). A recent survey of oxygenated sediments from the abyssal plains also found an abundance of Alveaolata, Euglenozoa, Heterokonta, and Rhizaria, and again, many of their closest relatives have parasitic lifestyles (485). Furthermore, diversity analysis revealed a highly diverse population of microbial eukaryotes in marine sediments (485), indicating that more efforts are needed to understand the factors controlling eukaryote diversity and function in sediments.

Viral abundance is relatively high in surficial sediments from both shallow- and deep-water environments (104). Both viral abundance and production positively correlate with their prokaryotic counterparts. Viral production occurs *in situ*, and the viruses do not appear to be introduced from attachment on falling particles. The amount of virus-induced prokaryotic mortality increases with water column depth, to 80% in open ocean sediments, indicating that the release of carbon from microbial cells through viral lysis is very important in deep-sea sediments. This means that lytic viruses are prevalent in deep-sea sediments, in contrast to other marine environments, such as hydrothermal plumes (see below) (266, 430, 609, 615). The viral shunt is estimated to account for ~0.37 to 0.63 Gt C $year^{-1}$ in the deep sea, providing organic carbon to fuel 35% of benthic metabolism.

Oceanic Crust

General physical and chemical characteristics. Oceanic crust (Fig. 2) comprises the largest aquifer system on Earth, with an estimated rock volume of 2.3×10^{18} m^3 and a fluid volume of roughly 2% of the total ocean (Table 1). Due to hydrothermal circulation (discussed in later sections), the entire volume of the ocean circulates through the ocean crust on the order of every 10^5 to 10^6 years (157), making this subsurface ocean within the oceanic crust a site of geologically rapid chemical exchange between the crust and the oceans, which has significant ramifications on global chemical cycles (26, 28). For example, the delivery of iron leached from oceanic crust into circulating fluids and injected into the water column is estimated to rival the input of iron from terrestrial runoff (605), which is significant since iron is an important requirement for photosynthetic organisms in the ocean. Fluid flow through the basaltic crust aquifer also appears to balance elemental budgets (sources and sinks) of Mg and Ca in the oceans (117).

The physical construction of oceanic crust is often described as three layers—the upper few hundred meters of fractured extrusive and intrusive basalts; a middle layer down to roughly 1.5 km below seafloor of sheeted dike complexes, which are essentially the solidified igneous "piping" through which magma travels; and a deeper layer to roughly 4-km depth of igneous crystalline gabbroic rock. The upper layer is characterized by extensive fracturing, roughly 10% porosity, and permeabilities on the order of 10^{-12} to 10^{-15} m^2 (11). These values tend to decrease with age of the ocean crust as fractures are filled in by compression or mineral precipitation, although there are exceptions.

New oceanic crust is formed along midocean ridges and at midplate hot spots, where interaction between Earth's magma and the lithosphere leads to the generation of new crustal material. Globally, an areal average of 3 km^2 $year^{-1}$ and a volume average of 21 km^3 $year^{-1}$ of new crust is formed on the seafloor (103, 437), although the production of new crust is an episodic process. Depending on the local sedimentation rate, oceanic crust can become buried with age as the oceanic plates move away from the midocean ridges. Roughly 2×10^5 km^2 of relatively young oceanic crust is exposed at the seafloor along midocean ridges (131). Furthermore, nearly 70% of the ocean seafloor is exposed or shallowly buried (<100 m of sediment) oceanic crust (Fig. 1). Recent estimates suggest that seamounts make up a combined area of nearly 30×10^6 km^2, a massive biome larger than the global sedimentary continental shelf biome (148). The eruptive rates of the oceanic crust correlate to some degree with the spreading rate of the plates along midocean ridges, although slower-spreading ridges are also driven by tectonic forces (185, 367). Fast- to intermediate-spreading ridges include the East Pacific Rise and the Juan de Fuca Ridge in the eastern Pacific and the Central Indian Ridge; the Mid-Atlantic Ridge is a slow-spreading ridge; and ultraslow-spreading ridges include the Southwest Indian Ridge and the Gakkel Ridge in the Arctic.

A cross section of oceanic crust reveals a number of different provinces, as recently reviewed elsewhere (491). Along the midocean ridge, the areas of intensive eruption and hydrothermal advection are known as the ridge crest axis. As the oceanic plate moves away from the midocean ridge, the ridge axis transitions into the young ridge flank environment, with crustal ages of roughly 1 to 20 Ma. The young ridge flank is often characterized by relatively rapid fluid flow of cooler fluids through highly permeable rock. Older ridge flanks (up to 65 Ma) are generally buried under a layer of sediment, with thickness depending on the local sedimentation rate, and fluid flow is generally reduced due to the formation of alteration minerals, which eventually seal open fractures in crust. Although local fluid flow fluxes are generally lower on the ridge flank than on the ridge axis, it is estimated that roughly 60% of heat loss and fluid flow occurs on the flanks (137). Within the ridge flank environment, fluid flow is driven by latent heat forcing and relatively small differentials in temperature and pressure, being confined by low-permeability sediment cover to recharge

and discharge in discrete, high-permeability locations of outcropping crust or seafloor fractures (158). These recharge and discharge areas on the ridge flanks are effectively windows of communication between fluids circulating in the ocean crust and the ocean basins (114). The three "bare" basalt outcrops on the eastern flank of the Juan de Fuca Ridge (384, 599) and the partially buried Tengosed and Dorado Seamounts on the Cocos Plate near Costa Rica (239) are some of the best-studied examples of this type of environment. At convergent margins, where one oceanic plate subducts beneath another, heating and melting of the subducting and overriding plates lead to the formation of a range of new crustal environments—the forearc volcanoes associated with the subduction trench (such as the serpentizing mud volcano South Chamarro Seamount near the Marianas Trench [383, 601]), back-arc spreading centers (such as the Manus and Lau spreading centers [170]) encouraged from the pull on the overriding plate from the subducting plate, and back-arc volcanoes further away from the trenches (such as the Suiyo Seamount on the Izu-Bonin Arc [567]). At midplate seamounts (such as the Loihi and Vailulu'u Seamounts [268, 511]), volcanoes are sourced from deeper, less degassed mantle, leading to lower gas contents in hydrothermal fluids and also to lower temperatures and sulfide concentrations in the fluids.

Though much of the ocean crust is composed of basalt (an iron-bearing silicate), there are compositional exceptions to this rule. For example, in some sections of the Mid-Atlantic Ridge, the mantle rock known as peridotite is exposed at the seafloor. Peridotite is enriched in iron and manganese and is very highly reactive compared to basalt. Importantly for microbiology, mantle rock can undergo reactions with heated seawater at depth; this process—serpentinization—results in the generation of large amounts of H_2 (82, 175, 280, 281, 360, 361, 594). Seafloor expression of such processes and the resulting distinctive microbiological communities can be seen at the Lost City hydrothermal field (Fig. 5H; see "Hydrothermal Vents—Vent Fluids and Hydrothermal Chimneys"). At the other end of the metal content spectrum is the fraction of the oceanic crust classified as felsic or andesitic, being composed of greater amounts of SiO_2 (>69%) than of basalt and peridotite. These rock types are most common at convergent margins, such as subduction zones, formed from the interaction of a dehydrating subducting oceanic slab and the overriding plate (440, 457, 538).

Fluid-rock interactions within the crust lead to the removal of some chemical species from the circulating fluids (in particular Mg, SO_4^{2-}, and O_2) and the introduction of other chemical species to the fluids (namely, reduced metals, H_2, sulfides, and silica). Abiotic synthesis of organic matter can also occur in the crustal environment from the reaction of CO_2 and/or NH_3 with metals in the crust. Holm and Neubeck (227) suggested that hydrogen cyanide (HCN), a suspected precursor for abiotic synthesis of organic molecules, can form in the basement environment, particularly at subduction zones and in ultramafic settings. In this scenario, HCN is formed from the reaction of CO and NH_3, which are in turn derived from CO_2 and NO_3 reacting with native Fe and Ni found in absorptive silicate minerals (i.e., clays). Subduction zones and ultramafic environments are ideal candidates for this reaction because they provide a gradient in pH, making the reaction more favorable (i.e., at subduction zones, a relatively low-pH basement slab is overridden by a mantle-rich plate above [227]). The abiotic synthesis of organics in basement environments offers the tantalizing suggestion that these environments could have supported breeding grounds for early life on Earth.

Metabolic reactions. Knowledge of metabolic reactions occurring in the oceanic crust is sparse, as accessing this environment is technologically challenging. The majority of information available derives from analyzing the quantity and speciation of chemical constituents within rocks or fluids collected from the subsurface or at the seafloor (e.g., see references 26, 474, 602, and 603), by making inferences from functional genes observed in environmental DNA (354), or from incubation of mineral substrates in the environment or in the laboratory as a proxy for natural processes (133, 134, 425, 540, 559). Additional *in situ* experimental techniques are in development, especially for the hard rock subsurface (159, 424, 425), which promise to deliver more information about processes occurring within the oceanic crust.

Considering that basalt (Fig. 5D and E), which makes up the majority of the oceanic crust, is comprised of roughly 9% FeO [Fe(II)] and 0.1% each MnO and S, metabolic reactions involving these compounds are likely candidates for supporting lithotrophic activity in oceanic crust (26). Fe cycling supports metabolic activity in basalts, both in the form of oxidation of reduced iron sourced from the basalt mineral and in the reduction of secondary iron oxides that are formed on basalts. Comparison of the redox state of iron in drilled subsurface ridge flank basalts from a variety of ocean basins indicates an increase in oxidation with age of the crust to about 20 Ma, corresponding to a global oxidation rate of $\sim 10^{12}$ mol Fe $year^{-1}$ in that interval (26). The microorganisms responsible for Fe oxidation on basalts are not entirely clear, although commonly occurring alpha (including *Hyphomonas* species)-, gamma (including *Marinobacter* species)- and zetaproteobacteria are potential candidates for mediating this process (134, 479). Fe(III)-reducing microorganisms, including *Shewanella frigimarina* and *Shewanella loihica*, have been cultivated from basalt enrichments (346) and from seamount sampling (183), and genes involved in iron reduction, mostly cytochromes, have been documented in surveys of environmental DNA from basalts (354). Activities and rates of Fe oxidation or reduction in basalts in the environment are very poorly constrained because appropriate assays are not available. Laboratory incubation experiments with ground basalt and Fe-oxidizing bacteria isolated from the deep sea indicated that <0.01% of the basalt glass was dissolved per day (as measured by silica release [132, 134]). It is also unknown how Fe-respiring microorganisms partition within the basalt environment, based on physical and chemical constraints such as the rate of Fe(II) flux from solid substrates, the concentration of oxygen, nitrate, or hydrogen, and carbon availability, to name a few factors. The rate of basalt oxidation appears to be dependent on temperature (61), with very slow kinetics at low temperatures, so cold-adapted Fe-oxidizing bacteria have a competitive advantage at low temperatures (132). The mechanisms used by Fe-oxidizing bacteria to acquire reduced Fe from the solid basalt substrate are currently unknown but may include dissolution promoted by the generation of acidic conditions or ligands (132).

Mn(II) oxidation is another metabolic process that is pro-

moted by basalt-hosted microorganisms (539, 541, 550), although no known Mn(II) oxidizers are capable of autotrophic growth. The *Bacillus* genus within the *Firmicutes* is the most frequently identified Mn-oxidizing group (123). Other common Mn oxidizers recovered from basalts include the gammaproteobacterial genera *Marinobacter* and *Pseudoalteromonas* and the alphaproteobacterial genus *Sulfitobacter* (354), as well as *Actinobacteria* and some Gram-positive bacteria.

On average, basalts contain roughly 0.1% sulfur in the form of sulfide (S), which could serve as an energy source for metabolic reactions (26). Furthermore, reaction of sulfate from seawater with autochthonous organic matter or H_2 in the crustal environment could fuel sulfate reduction. However, rates of S oxidation or sulfate reduction in the oceanic crust are poorly constrained. As with Fe speciation in basalts, analysis of S speciation in globally distributed basalt samples indicates a global oxidation rate of S of roughly 10^{11} mol S $year^{-1}$ within young oceanic crust (26). Genomic surveys of environmental DNA from basalts revealed the presence of genes required in dissimilatory sulfate reduction (354), although definite proof of the existence of common sulfate-reducing bacteria (SRB) or archaea in basalts is currently lacking. Species distantly related to known SRB of the *Deltaproteobacteria* have been documented in basalt communities (354, 479), but it is unclear if these species are sulfate reducers. Members of the *Epsilonproteobacteria*, commonly found in S-oxidizing environments (as described above), are detected infrequently in basalt microbial communities. Recent investigations using stable isotopes of sulfur in pyrite grains in subsurface basalts suggested the occurrence of active sulfate reduction under reducing conditions (12, 13, 314, 474).

Nitrogen (N) cycling in the oceanic crustal environment is a relatively unexplored topic of research in the deep sea. In general, basalts are not N rich, averaging about 2 ppm (353). Seafloor-exposed basalts are exposed to bottom water nitrate levels of roughly 40 μM, while nitrate is generally depleted in hydrothermal fluids that circulate through the crust, although the circulating fluids may contain ammonium. The capacity for N cycling on basalts is suggested from the available free energy of nitrate reduction coupled to Fe oxidation and from observation of N cycling genes in environmental DNAs from basalts (354). In particular, genes involved in the pathways for N fixation, ammonium oxidation, and denitrification have been observed, although N-cycling microorganisms have not been recovered equivocally from basalts. 16S rRNA gene clone libraries indicate the presence of *Planctomycetes* species on basalts that are phylogenetically related to known anammox bacteria (479), as well as species related to *Nitrosococcus* and *Nitrosospira* (356, 479), which are known ammonium-oxidizing bacteria, although the capacity for anammox or nitrification by these communities is currently unknown. Furthermore, denitrification is another potential process occurring on basalts, supported by evidence of denitrification genes in basalt environmental DNA (354) and enrichment experiments with cultivated Fe-oxidizing bacteria growing on basalt as a reduced Fe source with nitrate as an electron acceptor (134).

Metabolic processes that require gaseous substrates, such as H_2, CH_4, and CO, for energy generation in basaltic environments are also understudied at present. Fluid-rock reactions within oceanic crust are known to generate H_2 via serpentinization reactions, generating an energy-rich substrate which can be coupled to all known electron acceptors for energy generation; however, rates of H_2 oxidation in the oceanic crust have not been measured. Similarly, methanogenesis is suspected to occur on basalts in anaerobic niches, although definitive proof of this process is lacking. Enrichment experiments with basalts from the Arctic Ocean generated methane over time; however, no known methanogenic archaea were recovered from sequence analyses (346). Functional gene surveys with environmental DNAs from basalts indicated the presence of methanogenic genes (314, 354); however, no known methanogen-related 16S rRNA gene sequences were detected in the same samples (354). Functional gene analysis also indicated genes involved in bacterial aerobic methane oxidation (354), although this process has not been confirmed in the crustal environment.

The balance of autotrophic versus heterotrophic modes of life in basaltic environments is unclear. Using theoretical modeling, Bach and Edwards (26) predicted that aerobic and anaerobic (using NO_3) Fe and S oxidation could support $\sim 50 \times 10^{10}$ g C $year^{-1}$ of autotrophic primary production in oceanic crust, with H_2-based sulfate reduction and methanogenesis supporting an additional 1×10^{10} to 10×10^{10} g C $year^{-1}$ (assuming production of hydrogen from basalt reaction). Furthermore, these calculations suggest that H_2 reduction of Fe oxides, nitrate, and oxygen could also support 10^{10} to 10^{11} g C $year^{-1}$ each, assuming that available hydrogen and oxidized species are present (26). These autotrophic primary production values are similar in magnitude to the rate of heterotrophic organic consumption in sediments (26), indicating that autotrophic processes in the oceanic crust could be significant contributors to the global C cycle. A survey of environmental DNA from seafloor-exposed basalts revealed the presence of genes for proteins involved in carbon fixation processes (RubisCO + ATP citrate lyase) (354), providing further support for the existence of autotrophy in this environment. Further work is necessary to document the magnitude of autotrophy in oceanic crust environments.

Microbial distribution and diversity. There is somewhat more information available about the composition of microbial communities inhabiting crustal materials than on rates of processes, although linking phylogeny with function in the crustal environment is problematic because so little is known about this environment. Direct evidence of microbial life within crustal materials is a field of research that is only 20 years old, with the first visualization of DNA-stained material in rocks collected from the Costa Rica Rift (188). More recent surveys have documented biofilms of microorganisms coating the surfaces of seafloor-exposed basalts (Fig. 5D) (479). Additional studies have confirmed colonization of fresh mineral surfaces by microorganisms (133, 425, 517, 540). Further support for microbial activity in crustal materials derives from the observation of conspicuous "tubes" that are observed in basalt glasses, which are hypothesized to be generated by microorganisms growing into the surfaces of minerals (162, 163, 178, 179, 188).

From the few studies available, it appears that seafloor-exposed basalts host microbial communities with densities of 6×10^5 to 1×10^9 cells g^{-1} on the surfaces of the rocks (136, 354, 479). Few data are available to derive solid trends in

global density patterns on seafloor-exposed basalts related to age of the material, chemical composition, or other factors. Some evidence indicates that the density and diversity of microbial communities increase with age and alteration of the rock (478), although another study suggests that community density does not vary with age or water depth (136). The density of microbial communities residing in subsurface crustal materials is currently unknown. Furthermore, the surfaces of basalts can be colonized heterogeneously, as evidence suggests preferential growth in pits and grooves on the surfaces of minerals (133).

Seafloor-exposed basalt microbial communities are dominated by bacteria, based on quantitative molecular methods such as quantitative PCR with environmental DNA extracts from basalts and fluorescence *in situ* hybridization (FISH) (14) analysis of the surfaces of minerals (136, 479). Phylogenetically, the bacterial communities tend to be dominated by *Proteobacteria*, in particular the *Alpha-* and *Gammaproteobacteria* (Fig. 7) (136, 354, 479). *Actinobacteria* species are also common components of seafloor-exposed microbial communities, as are the *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, and *Planctomycetes* phyla (354, 356, 478, 479). Within the *Gammaproteobacteria*, many of the detected species are of unknown physiology, although some species group closely with known methane- and sulfide-oxidizing groups (478). Within the *Alphaproteobacteria*, many detected species group with chemoorganotrophic groups (478). Within the other detected phyla, the function of the observed species is poorly understood. Furthermore, the microorganisms responsible for iron oxidation in this environment are not obvious, as known marine Fe-oxidizing bacteria (i.e., *Zetaproteobacteria*) are rarely recovered in 16S rRNA gene clone libraries, although recent studies have enriched microorganisms from basalts that produce siderophores, enzymes involved in metal cycling (517).

The community composition of subsurface basalts in the oceanic crust is poorly known at present, largely due to an overwhelming lack of high-quality samples to investigate. Attempts to describe the microbial communities inhabiting deep oceanic crust, collected by oceanic drilling, are often hindered by contamination issues (312, 478). One recent survey for functional genes of anaerobic processes that are unlikely in contaminant material (i.e., anaerobic methane cycling) in subsurface basaltic environmental DNA indicated the presence of methanogen-like archaea within basalt veins (314). Given these limitations, microbial communities inhabiting deep oceanic crust have been inferred from studies of crustal fluids collected on ocean ridge flanks. The first such study revealed that a warm (~64°C), reducing, subsurface oceanic crust environment hosted a microbial community dominated by *Firmicutes* bacteria (Fig. 7) (93). Subsequent studies in this environment confirmed this observation (392, 425). The function of these *Firmicutes* organisms is currently unclear, as the species observed do not group closely with cultivated microorganisms, although it has been suggested that the microorganisms may be involved in nitrogen or sulfur cycling (93, 392). More work is necessary to define the range of microorganisms that exist in subsurface oceanic crust and to determine their metabolic processes.

The composition and function of archaeal, eukaryotic, and viral communities in oceanic crust are less well understood than those of their bacterial counterparts. In the few surveys that have been conducted on basalts, the archaeal communities tend to be dominated by members of MGI or MBGB of the *Crenarchaeota* (Fig. 8) (163, 346, 356, 551; B. Orcutt and K. Edwards, unpublished data). In contrast, studies of deep oceanic crust exposed to reducing fluids indicated the presence of more anaerobic archaea, such as *Archaeoglobus* and *Methanosarcina* (93, 425). The function of these archaea in oceanic crust is unknown. One recent survey of basalts and microbial mats from Vailulu'u Seamount found several fungal species hosted in these habitats (90). Furthermore, this study documented evidence that the fungi both produce siderophores known to be involved in increasing the bioavailability of Fe(III) and can slowly oxidize Mn(II), indicating a role of fungal species in metal cycling in these habitats.

The succession pattern of colonization of basalts has been examined in a few cases and indicates that the composition of microbial communities on seafloor-exposed basalts appears to vary with age (346, 356, 478). For example, microorganisms that form twisted stalks of Fe oxyhydroxides, i.e., microaerophilic Fe oxidizers, are more prevalent on young basalts (540, 551). One hypothesis is that fresh basalt surfaces are first colonized by chemolithoautotrophic microorganisms, such as S and Fe oxidizers, which take advantage of the juxtaposition of reduced mineral substrates and the surrounding oxic seawater to fuel metabolic activity while fixing carbon (26, 131, 479). The production of secondary mineral oxides and organic carbon on the surfaces of the minerals, along with the existence of small-scale topography in the form of pits and fractures in the rock surface, leads to the formation of microniches on the basalt which can be exploited by different microorganisms. Depending on metabolic rate and fluid flow, some microniches may become anoxic, allowing anaerobic reactions to occur. However, more recent investigations suggested that seafloor-exposed basalt microbial biofilms and mineral crusts are instead seeded from hydrothermal inputs, based on colonization experiments revealing established biofilms on basalts without corresponding pitting or etching into the host basalt (540).

Seafloor-exposed basalts (Fig. 5D and E) harbor some of the most diverse bacterial communities on Earth, in terms of both the number of different species hosted on basalts versus other habitats and the number of different phyla (479). The underlying mechanism explaining this high degree of diversity on basalts is not certain, although it is inferred that the plethora of potential redox reactions that could be supported by basalts in juxtaposition with oxidized seawater, involving Fe, Mn, N, S, and C cycling, supports a wide range of possible metabolic processes and microniches to exploit (479).

Hydrothermal Vents—Vent Fluids and Hydrothermal Chimneys

Hydrothermal vents, first discovered off the coast of the Galapagos in 1977, represent conspicuous, biological oases on the dark seafloor that are underpinned by chemosynthetic production (Fig. 2 and 5) (91, 250). Hydrothermal vents are characterized by the emission of thermally charged fluids from the subsurface, often accompanied by the formation of hydrothermal mineral deposits in the form of chimney structures surrounding advecting vent fluids and/or the deposition of mineral particles following mixing of vent fluids with seawater (Fig. 5).

Considering that hydrothermal fluids emanate from the subsurface, these environments are considered "windows into the subseafloor" (114). Thorough reviews of hydrothermal vent ecology have been published previously (529, 572). In this section, we discuss biogeochemical and microbial ecological patterns found both in the hydrothermal vent fluids and within the hydrothermal chimneys.

General physical and chemical characteristics. Hydrothermal vents at the seafloor often occur at or near tectonic and/or volcanic boundaries, such as midocean ridges and subduction zones, or at midplate hot spots of volcanic activity that occur at seamounts. Generally, most hydrothermal venting occurs in areas with little sediment cover on young oceanic crust, although there are also instances of hydrothermal vents in sediment-hosted systems, such as Guaymas Basin in the Gulf of California (502) and Middle Valley on the landward eastern flank of the Juan de Fuca Ridge (197). Both of these systems are sediment covered due to their close proximity to land and sediment loading from continental sources. Fluids that circulate through the ocean crust near vents undergo heating and water-rock reactions, resulting in a net gain in thermal energy and gaseous compounds (such as methane and carbon dioxide) from the underlying magma. These evolved hydrothermal fluids, which are chemically reduced in relation to the source seawater, vent at the seafloor, essentially acting as agents of heat and mass exchange between the Earth's interior and the oceans. The volume of high-temperature hydrothermal fluids emitted through these chimneys is a small fraction of the total hydrothermal flux, which also includes cooler, off-axis flow. Chimney and ridge crest hydrothermal flow contributes 425×10^{16} g water per year, which is 1/10 the mass of total hydrothermal flow (185). However, even though the mass of hot hydrothermal flow is relatively small, it carries nearly half of the total heat flux from hydrothermal systems.

The venting fluids exhibit a range of temperatures, with relatively cool temperatures only a few degrees above ambient to less than 100°C at diffuse flow vents (evidenced by shimmering water due to the density differences between the mixing water masses), white smoker vents with warmer advecting fluids (100 to 300°C; the white "smoke" results from the precipitation of barium, calcium, and silica minerals), and black smoker vents with temperatures of up to 400°C (where the black appearance results from the precipitation of metals and sulfide minerals within the vented fluids) (Fig. 5F and G). Hot, chemically reduced vent fluids that are emitted into the overlying cool, oxidized water column undergo mineral precipitation and oxidation as a result of mixing and cooling. At the venting orifice at the seafloor, mineralization of metal sulfide chimney structures occurs, templated over a matrix of anhydrite (calcium sulfate) (554). Chimney deposits form rapidly, on the order of days to weeks (210), although the longevity of individual chimney structures varies over a range of timescales, with some vent structures estimated at thousands of years old (175). Alkaline vent fluids, such as those emitted at the ultramafic rock-hosted Lost City vent field in the mid-Atlantic, lead to the formation of carbonate chimneys (Fig. 5H) (280, 281), while acidic fluids, such as those emitted at the overwhelming majority of studied sites, result in sulfide chimneys (Fig. 5F and G). In most cases, in addition to the vertical movement of vent fluids due to positive buoyancy, horizontal movement of fluids through porous features in the chimney structure result in complex, multiple fluid emission sites along a chimney that can be exploited by microorganisms. Horizontal venting can lead to the formation of beehive and flange structures on the sides and tops of chimneys (109). Despite the visual prominence of chimney structures and the dramatic presence of white and black "smoke" at these high-temperature vent sites, cooler (less than 100°C) emission of diffuse fluids is also prevalent at seafloor hydrothermal systems. There is evidence that low-temperature, diffuse fluid venting may be a much more important process, quantitatively, than high-temperature venting in terms of chemical fluxes and habitat size (495); however, diffuse low-temperature venting is poorly studied by comparison.

The chemistries of vent fluids (Table 6) are largely dependent on the composition of the source rock (see "Oceanic Crust") and on the temperature and duration of reactions. Oxygen is removed quickly during heating and water-rock reactions. Sulfate is generally removed from the circulating fluid due to the precipitation of anhydrite ($CaSO_4$) upon heating and via reaction with crustal minerals to form sulfides. Magnesium is rapidly stripped from the source seawater, whereas fluids tend to gain silica from hydrothermal alteration reactions. Iron-silicate minerals form during reaction, and vent fluids become enriched in reduced iron, hydrogen, and sulfide gases, depending on the temperature of the reaction (8, 248, 598). Well-studied basalt-hosted vent sites include the Loihi Seamount off Hawaii, the Juan de Fuca Ridge off the coast of Washington State, the East Pacific Rise offshore Central America, and many vent locations along the Mid-Atlantic Ridge (49, 171). In contrast, other vent sites—including the Rainbow vent field (169) and the Lost City field (280) on the Mid-Atlantic Ridge—are hosted in mantle-like crust (see "Oceanic Crust"). In addition to the presence of high H_2 levels, these vent fluids contain relatively high concentrations of organic carbon, including formate and acetate, and stable carbon isotopic evidence suggests that the formate is produced abiotically (305). Hydrothermal vent fluids in more silica-rich settings at convergent margins, such as back-arc basins, have more heterogenous chemistries that are a consequence of a complex suite of thermal and dewatering reactions during subduction of sediments and crust (514). Subduction-related venting is most well known in the western Pacific Ocean, where the Pacific Plate is subducting beneath the Philippine Plate, but it is also known in other places, such as the East Scotia Ridge in the South Atlantic and the Chile Triple Junction in the South Pacific.

Metabolic reactions. Anaerobic microbial processes tend to dominate within high-temperature vent fluids, although the transition to aerobic processes may occur over short spatial scales due to diffusive and advective fluid mixing (360, 362, 552). Methanogenesis, based on H_2 oxidation coupled to CO_2 or SO_4^{2-} reduction, is suggested as a dominant anaerobic process within the high-temperature vent fluids (generally above 45°C) at basalt-hosted vents, whereas sulfide oxidation, metal oxidation, and methane oxidation become more important in the cooler venting fluids, as in hydrothermal plumes (359, 362, 552). In ultramafic rock-hosted vents, sulfur cycling processes are less important, with hydrogen and methane metabolism dominating (360).

It has been suggested that a sizeable fraction of biomass in

hydrothermal vent fluids is generated from chemolithoautotrophy (250), although direct quantification of primary production rates in vent fluids has not been explored thoroughly (267). Among anaerobic reactions (which are also generally mediated by thermophilic or thermotolerant species), methanogenic archaea that consume CO_2 are autotrophic candidates (362), although many known thermophilic archaea are heterotrophic (reviewed in reference 35). For aerobic mixing regimens, a number of thermophilic autotrophic sulfide oxidizers are also known (76, 529). Thermodynamic modeling suggests that sulfide oxidation accounts for more than 90% of available energy in some vent fluids (362). Theoretical calculations suggest that ultramafic rock-hosted vent systems can support roughly five times more primary production per volume than basalt-hosted systems (i.e., 35 versus 189 mg of dry weight biomass per kg of hydrothermal fluid in basalt-hosted versus ultramafic rock-hosted systems [360]). This is suggested to be related to the larger proportion of hydrogen in ultramafic rock-hosted venting fluids, although the overall energy yields are roughly the same between the two venting end members (360).

One intriguing issue with regard to microbial activity in hydrothermal fluids is the upper temperature limit for life. Among other biochemical consequences, high temperatures lead to changes in the structure and composition of proteins and lipids to compensate for increasing fluidity (226), higher G+C contents of DNA due to increased hydrogen bonding strength (182), and the expression of heat shock proteins (285, 301, 560). Currently, 122°C is the highest known temperature at which microorganisms have been grown in the laboratory, and high hydrostatic pressure is necessary to prevent boiling (272, 515, 531). Some have suggested that the upper temperature limit for life might be closer to 150°C (220, 225), and limited evidence suggests that microorganisms may be able to at least persist at higher temperatures (494). Considering that hydrothermal chimneys can have interior portions that sustain temperatures above 150°C, it remains possible that higher temperature limits for life could be observed in the future.

Somewhat surprisingly, there is also mounting evidence for nonsolar phototrophic metabolism occurring at hydrothermal vents. The first evidence for this process came from the observation of novel photoreceptors in vent macrofauna that are never exposed to sunlight (574). Further efforts confirmed that infrared and even visible light is generated from black body radiation from the high-temperature venting fluids at the seafloor (573). More recently, an anaerobic phototrophic sulfur-oxidizing bacterium designated GSB1 was isolated from vent fluids at the basalt-hosted East Pacific Rise vents (40). This bacterium uses bacteriochlorophyll *c* and the carotenoid chlorobactene to harness light energy, and the species is phylogenetically related to the green sulfur bacteria *Chlorobium* and *Prosthecochloris*. Additionally, genetic surveys of the inside an active vent for genes that encode the carbon fixation protein RubisCO recovered sequences most closely related to cyanobacteria and green sulfur bacteria, both of which are phototrophic groups, further indicating the presence of microorganisms utilizing photoradiation for energy (585). Future work is necessary to identify how prevalent phototrophic forms of metabolism may be at hydrothermal vents.

Metabolic reactions within hydrothermal chimneys mirror,

TABLE 6. Average chemical compositions of vent fluids from representative hydrothermal venting environments[a]

Parameter	Value or description for ocean site										
	Mid-ocean ridge	Baby Bare	Back-arc basin	Chamorro mud volcano	Rainbow vent	Lost City	Guaymas Basin	Loihi Seamount	Seawater
Ocean basin	Atlantic, east Pacific	Pacific	Pacific	Pacific	Atlantic	Atlantic	Pacific	Midplate, Pacific	Pacific
Crust composition	Basalt	Basalt outcrop in sediment	Basalt or ultramafic rock	Subduction, ultramafic rock	Ultramafic rock	Ultramafic rock	Sediment	Basalt	
Temp (°C)	≤405	~25	278–334	25	365	≤91	100–315	13–52	
pH (at 25°C)	2.8–4.5	8.3	<1.0–5.0	12.5	2.8	10–11	5.1–5.8	5.1–7.0	7.9
Cl concn (mM)	31–1,276	554	261–810	510	769	562	422–685	538–560	542
Na concn (mM)	11–1,008	473	215–605	610	567	491–497	323–574	463–477	467
SO_4^{2-} concn (mM)	0	17.8	0	28	0	1.03–4.1	0	26.8–29.3	28.1
Mg concn (mM)	0	0.98	0	<0.01	0	<1.03	0	48.2–55.8	52.6
Si concn (mM)	3.8–17	0.36	14–16	0.07	6.9	~0.19	9.5–13.5	0.37–4.7	0.19
Ca concn (mM)	4–112	55.2	6.7–91	0.3	68.7	<30.8	164–263	10.5–22.4	55.2
K concn (mM)	1.2–60	6.88	10.7–81	19	20.5		13.8–50.4	11.8–13.5	10.1
Ba concn (μM)	1.68–19	0.43	6–103	0.4	>68.7	<0.066	>12.3	0.64–0.90	0.15
H_2S concn (mM)	~20	1.3	1–13	0.25	1.025	<1.03–16.5	1.13–6.13	≤0.47	
H_2 concn (mM)	0.00051–39		0.036–0.51	45	13.3	ND			2.5
CO_2 concn (mM)	3.6–41	0.85	15–205	2	ND	Below detection limit			
CH_4 concn (mM)	0.0072–2.6		0.0051–0.062	0.22	0.13–2.3	1.03–2.05			
NH_3^+ concn (mM)	0.666	0.076		2			5.74–16.0	2.95–7.2	0.0003
Fe concn (μM)	7.2–19,168	<0.05–6	13–2,563	2	24,600		0–185	64–1,497	0.001
Mn concn (μM)	60–3,383	2.9–4	13–7,278	0.01	2,306		210.3–42	2.8–49.6	0.001

[a] Based on data from references 185 and 553. Seawater and Baby Bare data are from references 384 and 605, back-arc basin data are from reference 535, Rainbow vent data are from reference 83, Guaymas Basin data are from reference 580, and Loihi Seamount data are from references 191 and 498. Concentrations for background seawater are provided for comparison. ND, not done.

to some degree, the processes that occur in the fluids and are related to temperature, oxidation state, and mixing. Within active hydrothermal chimneys, the zonation of microbial metabolism varies depending on diffusion and advection processes across the chimney walls affecting the temperature, pH, and chemical concentrations (362, 552). As in the venting fluids, sulfate reduction and methanogenesis predominate in the hot anoxic sections of the chimney, while a range of aerobic processes can occur further from the venting source (362). In inactive chimney deposits, where fluid venting has ceased, sulfide oxidation and, to some degree, metal cycling are the dominant microbial reactions (470; B. M. Toner et al., submitted for publication). In Fe and Mn oxide mats that form around diffuse flow venting, metal-cycling types of metabolism dominate (105, 144, 277).

Microbial distribution. *In situ* collection of vent fluids and active hydrothermal chimney samples for microbiological analysis can be challenging, due to the hot, corrosive, and potentially ephemeral conditions of the environments. Within the past 2 decades, scientists have mounted rapid response research programs to visit hydrothermal vent sites as soon as possible after documented volcanic or tectonic activity in an effort to capture the microbial communities associated with recent venting and to establish baselines for community dynamics and maturation (31, 142). Researchers have employed colonization chambers or unique fluid collection devices to obtain samples. One approach to collecting high-temperature venting fluids is via insertion of a sampling tube below the seafloor, either by penetration of the seafloor with a heavy probe with embedded sampling lines (230, 232, 252) or by inserting the open end of a sampling device directly into a vent or shimmering water (73, 441) or into a crevice in the seafloor (277). This is done in an effort to avoid entrainment of seawater in the fluid samples. The microbial communities inhabiting active hydrothermal chimneys have also been evaluated by obtaining pristine samples with colonized structures that form on *in situ* growth chambers placed over the orifice of an active vent (467).

The microbial communities in high-temperature vent fluids in basalt-hosted systems tend to be enriched in sulfur- and H_2-respiring *Epsilonproteobacteria* and in thermophilic methanogenic and sulfate-reducing *Euryarchaeota* (Fig. 7 and 8) (76, 332, 422, 423, 467). For example, one study targeting hydrothermal fluids circulating below the surface at the back-arc Suiyo Seamount off Japan recovered different groups of *Epsilonproteobacteria* and *Methanococcales* in 16S rRNA gene clone libraries (217). One of the most thorough examinations of hydrothermal fluid microbial communities comes from a combination of culture-dependent and -independent analyses of fluids from basalt-hosted vents on the Juan de Fuca Ridge following well-documented eruptive events (230–233). In the first set of studies conducted at Axial Volcano, microbial diversity was higher in particle-rich vent fluids. Within the bacterial community, the *Epsilonproteobacteria* were an abundant group (roughly one-third of 16S rRNA gene environmental clones), followed by *Gammaproteobacteria* (roughly one-fourth of clones) and *Deltaproteobacteria*, specifically *Desulfurobacterium* relatives and the novel candidate phyla WS6 and ABY1 (230). Within the *Gammaproteobacteria*, diversity was low, with most environmental 16S rRNA gene clones associated with a few chemosynthetic endosymbiotic species and with *Marinomonas* and *Alteromonas* (230). Some species of *Planctomycetes* and the *Cytophaga-Flexibacter-Bacteroides* (CFB) group were also evident in the vent fluid samples (230). In a further study of hydrothermal fluids from Axial Volcano, using massively parallel sequencing of a short, hypervariable segment of the 16S rRNA gene, earlier conclusions that *Epsilon-* and *Gammaproteobacteria* are the dominant groups of bacteria within these vents were confirmed (233). Among archaea in Axial Volcano vent fluids, methanogens appear to dominate, based on both cultured isolates and phylogeny of 16S rRNA gene sequences from the environmental samples (231). In particular, *Methanococcales* and *Thermoplasmales* relatives are abundant, and it was suggested that the *Thermoplasmales* may be associated with particles in the vent fluids for their life cycle (231). The largely uncultivated MGI and MGII clades of the *Crenarchaeota* and *Euryarchaeota*, respectively, were also present in the examined fluids, although it is thought that these archaea represent seawater entrainment or influence (231). Both traditional (231) and massively parallel (233) sequencing techniques indicate that the archaeal community is significantly less diverse than the bacterial community in hydrothermal vent fluids. Additionally, studies at the Axial Volcano have documented an increase in bacterial diversity in diffuse-flow venting fluids with age since an eruptive event (230, 231), although additional studies at these sites have observed relatively stable intravent community differences over time (423).

A set of studies from vent sites on the eastern flank of Juan de Fuca Ridge, centered around the Baby Bare basalt outcrop diffuse hydrothermal (~25°C) vents and an instrumented observatory that targets deeply buried (200 to 300 m) basement hydrothermal (~65°C) fluid flow a few kilometers away (93, 232, 392, 425), found some similar patterns in microbial ecology in basalt-hosted hydrothermal fluid. As before, thermophilic bacteria related to *Desulfurobacterium* and *Epsilon-* and *Deltaproteobacteria* were abundant in 16S rRNA gene clone libraries, although *Thermotogales* and candidate phylum OP8 were also detected (232). *Thermococcales* archaea were again observed in cultures and 16S rRNA gene clone libraries, although the hydrothermal fluids here also had a larger proportion of *Crenarchaeota* than had been observed previously (232). Additionally, sediment-influenced hydrothermal fluids appeared to have a somewhat different community membership, with domination by green nonsulfur bacteria. In other studies at the instrumented basement observatory, 16S rRNA gene clone libraries indicated an abundance of *Firmicutes* bacteria (93, 392, 425), although the metabolic capabilities of the detected microorganisms were somewhat speculative, maybe involving sulfate reduction or ammonium oxidation.

In contrast, studies of microbial ecology in cooler, Fe-rich, diffusely venting environments, such as at Loihi Seamount offshore Hawaii and around Suiyo Seamount, indicate the predominance of potentially Fe-oxidizing microorganisms belonging to the recently described *Zetaproteobacteria* (105, 143–145, 277, 386, 454). Cultured representatives of the *Zetaproteobacteria* are known as microaerobic, neutrophilic, Fe-oxidizing bacteria that produce conspicuous twisted stalks of iron oxyhydroxides (145). In addition to their presence in microbial mats and diffuse flow vents, zetaproteobacterial sequences have also been detected with relatively high frequency in some

inactive massive sulfides (Fig. 5A) (Toner et al., submitted). However, other relatively low-temperature mats around the Vailulu'u Seamount are instead apparently dominated by *Alpha-*, *Gamma-*, or *Epsilonproteobacteria* (517). Low-temperature (8°C) venting fluids at the Clueless field on the southern Mid-Atlantic Ridge are also dominated by *Gamma-*, *Epsilon-*, and *Deltaproteobacteria*, with no detectable archaea (441). Both of these environments have higher sulfide concentrations than those observed in the *Zetaproteobacteria*-dominated environments, indicating that sulfide and Fe availability is a driving factor in determining microbial community composition in lower-temperature vents and mats in hydrothermal systems.

The microbial communities inhabiting hydrothermal chimneys resemble, to some degree, the communities observed in the venting fluids (Fig. 7 and 8). One of the first studies conducted on a black smoker vent at the basalt-hosted Snake Pit site on the Mid-Atlantic Ridge revealed an abundance of *Aquificales* and *Desulfobacterium*—hydrogen- and/or sulfur-metabolizing members of the *Epsilonproteobacteria*–-in 16S rRNA gene clone libraries (467). Among the archaea that colonized the chimney structure, the majority (>90%) of 16S rRNA gene sequences were affiliated with the thermophilic orders *Archaeoglobales* and *Thermococcales*, which are known to include thermophilic sulfate reducers and iron oxidizers (467, 512). Further studies with vent growth chambers indicated that archaea composed up to one-third of the microbial community in chimneys at basalt-hosted East Pacific Rise vents, dominated by the thermophilic heterotrophic methanogenic and sulfate-reducing archaea *Thermococcales*, *Methanopyrus*, *Pyrodictum*, and *Ignicoccus* (408). The relative abundances of the various archaeal groups have been shown to vary with the age of the chimney structure (408, 467), although the patterns of community development are currently unclear. For example, in one study conducted at 13°N at East Pacific Rise, heterotrophic microorganisms were the first colonizers of a newly formed chimney structure, followed by in-growth of chemolithoautotrophs (408), whereas autrotrophs were the first colonizers of chimneys formed over a vent at the Mid-Atlantic Ridge (467). In a colonization experiment at chimneys with different ages, chemolithoautotrophic archaea related to *Ignicoccus* organisms and their *Nanoarchaeum* symbionts dominated the initial colonization of high-temperature chimneys, whereas heterotrophs related to *Thermococcales* were more abundant at mature vents (358). This study supports the hypothesis that initial chimney colonizers are autotrophic, being replaced or joined by heterotrophs within days to hours of chimney formation.

In situ colonization experiments with hydrothermal chimney sulfides revealed that chemolithoautotrophic Fe-oxidizing bacteria were the predominant early colonizers of the mineral surfaces, preferentially colonizing pits and pores, which has been interpreted as a mechanism for targeting microniches where local oxygen concentrations can be drawn down to levels that make biotic Fe oxidation competitive against abiotic reactions (132, 133). These colonization experiments also revealed higher densities of microorgansisms on minerals with higher reactivities of Fe, indicating the role that abiotic kinetics has in selecting for microbial communities in hard rock environments (133). The cell densities on the reacted chimney sulfides were on the order of 10^5 cells mm^{-2}. Further studies with sulfides from an active high-temperature chimney revealed that the microbial communities were dominated by bacteria and that diversity levels decreased with increasing oxidation of the chimney material (470). In addition to *Zetaproteobacteria*, which have been documented on fresher sulfide material, species related to the *Gammaproteobacteria* grouping of *Marinobacter* and *Halomonas* are commonly found in 16S rRNA gene surveys of sulfide materials with a range of oxidation states (134, 470). More recent analysis of vent chimneys surrounding chloride-enriched or -depleted vents at the Brothers Volcano on the Kermadec Arc revealed that archaeal communities do not appear to vary with chloride influence, being dominated by marine benthic group E, while the bacterial communities do appear to vary with chloride enrichment (534).

In 2000, a unique hydrothermal vent field was discovered 15 km away from an active spreading center (280). This vent field, dubbed "Lost City," is formed from serpentinization reactions occurring within the mantle-like crust on which this site is hosted (also see "Oceanic Crust"). The hydrothermal fluids at Lost City are cooler (35 to 90°C) and alkaline, and the chimneys are composed of carbonate, not sulfides (Fig. 5H) (280, 281). Hydrothermal fluids at Lost City have high concentrations of methane and hydrogen, and their microbial communities are dominated by archaea with a low degree of diversity (63, 64, 493). Active carbonate chimneys are populated almost exclusively by a group of *Methanosarcinales* archaea which have not been observed outside Lost City, while extinct chimneys are dominated by archaeal members of the so-called ANME-1 group which are thought to be involved in the anaerobic oxidation of methane, although their potential for other metabolic pathways is debated (64). Recent deep sequencing efforts demonstrated that ANME-1-type sequences form a rare biosphere in the active chimneys, and likewise with the Lost City *Methanosarcinales* in the extinct chimneys, providing one of the first clear observations of ecological succession of rare biosphere members (63). Although lower in proportion than archaea, bacterial communities at Lost City are comprised of *Gammaproteobacteria*, *Epsilonproteobacteria*, *Nitrospira*, and *Planctomycetes*, which are suspected to play roles in aerobic methanotrophy, sulfide oxidation, nitrite oxidation, and anammox, respectively (63). It has been suggested that the high pH in the Lost City vent fluids is a driving factor in the low microbial diversity of this system (360). The observation of these archaeal and bacterial groups suggests that methane, H_2, S, and N cycling processes are the primary types of metabolism at this unique hydrothermal site. Given the global distribution of mantle-like crust, it is predicted that other hydrothermal systems like Lost City exist, but no additional sites have been seen to date. However, chemical signals highly indicative of a Lost City-like hydrothermal field were recently discovered along the Mid-Cayman Rise (184).

Hydrothermal Plumes

General physical and chemical characteristics. Hydrothermal vent plumes are neutrally buoyant masses of water in the deep ocean that originate from the mixing of positively buoyant hydrothermal vent fluids with seawater (Fig. 2). These plumes have subtle yet distinct chemical and physical differences from the surrounding seawater. The source hydrother-

mal vent fluids (see "Hydrothermal Vents—Vent Fluids and Hydrothermal Chimneys") are akin to terrestrial "hot springs," where seawater heated in the subsurface advects from fissures due to its lower density (from the higher temperature). Vent fluids, which as discussed above are often rich in reduced chemicals such as metals, hydrogen, methane, and sulfide, mix with seawater, and some of these reduced compounds may become oxidized and/or precipitate out of solution. Depending on the chemical compound, this can occur either immediately in the near-field plume environment or later during lateral dispersion of the plume. Thus, plumes are dynamic, distinct, and energetically rich environments due to the juxtaposition of oxygenated seawater and the reduced substrates that are otherwise scarce in oxygen-rich deep-sea waters. Table 6 provides an overview of the chemistries of some well-studied vent fluids. It is important that while some generalities about plumes can be made, vent chemistries vary to significant degrees from site to site (Table 6) and over time, depending on a variety of factors, including the underlying geology and the tectonic setting (579). Hence, the biogeochemical and microbiological features of plumes also vary temporally and spatially. For example, following signals of eruptive submarine volcanic events, rapid response sampling campaigns to hydrothermal vent sites have documented significant increases in volatile compounds, such as hydrogen, carbon dioxide, methane, and sulfide gases, within plumes (74, 108, 282, 320, 578).

When hydrothermal fluids are injected into the water column, they are less dense than the surrounding seawater because of higher temperature, and hence the fluids rise as they mix with the cooler seawater. This water mass is referred to as the rising or buoyant plume, and it continues to rise until the original vent fluids are diluted enough to achieve the same density (and neutral buoyancy) as the seawater. Neutral buoyancy is typically reached about 150 to 300 m above the vent source. After reaching neutral buoyancy, plume water masses disperse along density gradients in the water column following ocean currents. In addition to currents, the dispersal patterns of plumes are dependent in part on local bathymetry (i.e., the depth and shape of axial valleys or calderas). In some well-studied plume settings, the neutrally buoyant plumes have been traced up to 15 km away from the vent source, based on transmissivity of the plume water (a measurement of particulate concentration, which is elevated in plumes due to the oxidation and precipitation of reduced metal compounds) or by thermal temperature anomalies, depending on the strength and intensity of venting (185). Also, elevated methane concentrations can be traced up to 20 km from a venting source (612). Remarkably, measurement of helium isotopes, a conservative tracer of venting, has revealed signals of plume movement hundreds of kilometers away from venting sources, for example, on the East Pacific Rise (344) and the Loihi Seamount, off the coast of Hawaii (343). Considering the lateral extension of plumes, which can spread out over several tens of kilometers, hydrothermal plumes comprise a relatively vast habitat within the dark ocean. Additionally, the entire volume of the ocean passes through nonbuoyant plumes approximately every 4,000 to 8,000 years; therefore, chemical dynamics within plumes can have important consequences for compounds with shorter oceanic residence times, such as vanadium, phosphorus, and uranium (137).

Metabolic reactions. The most well-studied microbial processes occurring in plumes revolve around chemosynthesis based on mixing between reduced compounds (hydrogen, methane, ammonium, and reduced sulfur) and seawater; metal cycling; and the interactions between carbon, metals, and microbes in plumes. The highest potential energy source for fueling autotrophic growth in hydrothermal plumes is oxidation of elemental sulfur and sulfide precipitates, followed by H_2 oxidation, methanogenesis, and methanotrophy (359). Ammonium oxidation also provides an excellent energy source for autotrophic production (302, 303), and Fe and Mn oxidation has been demonstrated in plumes, although, as stated above, the latter is known only as a heterotrophic process (122).

The importance of chemosynthetic processes in hydrothermal plumes was demonstrated almost 30 years ago for plumes around the Galapagos Rift, with observations of ATP concentrations in plume fluids that were more than 300 times background seawater levels and with measurements of $^{14}CO_2$ uptake rates in plume fluids (269). The demonstration of chemosynthesis in plumes originally led to the hypothesis that chemosynthesis supports the symbioses associated with vent macrofauna, a relationship that is now well established (572). Chemosynthetic activities vary over the course of a plume's evolution, but considering the lateral extent of plumes over tens of kilometers, chemolithoautotrophy in plumes likely provides a significant amount of carbon to the deep oceans (43, 557).

Aerobic methane cycling in the plume environment is known to occur in plumes 2 to 15 km away from a known venting source (100, 106). Methane oxidation in plumes can contribute a significant fraction of carbon compared to the surface organic carbon flux to plume depths, with a turnover time for CH_4 on the order of days to weeks (106), although another study observed methane turnover times of 2 to 6 months (568). These differences can be attributed to the differences between the hydrothermal systems. The lower turnover rates are indicative of seamounts with a caldera, where methane can build up and be cycled within the low-oxygen caldera waters before rising higher into the oxidized water column. In support of this observation, methane turnover in plumes above gas cold seeps—where methane is slowly released into the water column from nonhydrothermal processes (see "General physical and chemical characteristics" under "Marine Sediments")–occurs on even slower timescales. For example, turnover times of 1.5 years have been estimated for methane in plumes above cold seeps in the Eel River Basin off the coast of northern California (570). In contrast, shorter turnover times are indicative of vent fields at spreading centers, where fluids are ejected directly into the oxidized water column and can therefore be oxidized more quickly.

NH_4^+ oxidation is also prevalent in hydrothermal plumes. A removal rate of 15 nM NH_4^+ day^{-1} has been observed for incubations carried out at 200 atm with plume samples from the Endeavor Segment of the Juan de Fuca Ridge (99). Similar experiments conducted at 1 atm yielded lower NH_4^+ removal rates. Other studies conducted with plume fluids from the same site measured higher NH_4^+ removal rates of up to 91 nM day^{-1}, accounting for 92% of net NH_4^+ removal in the plume (302). In the Endeavor plume samples, the production of organic carbon from ammonium oxidation provided 0.6 to 32 mg

C m^{-2} day^{-1} (when also including production from methanotrophs [106, 303]), which is equivalent to 133 to 3,400% of the surface-derived organic carbon flux reaching plume depths (302). Therefore, aerobic methanotrophy and ammonium oxidation in plume environments provide more carbon to the local ecosystem than the photosynthetically produced organic carbon falling from the photic zone.

Sulfur oxidation in plumes is the most energetically favorable metabolism, especially in the buoyant plume, where this metabolism dominates activity (359). However, field measurements of sulfur oxidation activity in plume samples are rare due to the difficulty of sample processing before abiotic and biotic oxidation occurs. One study suggested that 10 to 20% of sulfur measured above the caldera of the Axial Volcano was still present 7 months after an eruption (153). Using *in situ* methods, HS^- removal rates of 2 to 16 μmol $liter^{-1}$ day^{-1} were estimated (347). Laboratory experiments with *Sulfurimonas autotrophica* and *Sulfurovum lithotrophicum*, both of which are *Epsilonproteobacteria* commonly found in hydrothermal vents, demonstrated extremely high rates (10^6 to 10^7 μmol S oxidized $liter^{-1}$ day^{-1}) of enzyme activity for SOR:ferricyanide, a sulfite-oxidizing enzyme (521). These activity rates are much higher than those few measurements from the field, and future work is needed to understand the rates of sulfur oxidation *in situ* in the hydrothermal plume environment. Furthermore, this and other culture studies have demonstrated the capability of hydrogen oxidizers, such as *Nitratifactor salsuginis*, to also oxidize sulfite (500, 521), indicating that some microbes are capable of multiple types of metabolism.

Microbial cycling and scavenging of metals in the plume habitat are among the hallmark features of this biotope, with important implications for many global elemental cycles (96). Given that the predicted global hydrothermal fluid flux to the deep ocean, including both advective inputs and diffusive flow on axial ridge flanks, is equivalent to that of rivers to the surface ocean (185), the input of metals from hydrothermal sources is nontrivial. As an example, dissolved Fe in plumes accounts for ~20% of the deep ocean budget (43). Additionally, Fe particles in plumes bind V, As, Cr, Mo, and P and therefore act as a sink for these elements on the same scale that they are added to the oceans from riverine inputs (137). Microbially mediated metal oxidation in plumes leads to the formation of distinct morphologies of oxidized metal particulate matter, often in the form of Fe and Mn oxyhydroxides (94, 98). Biogenic mineralized capsules and other morphological forms of metal oxyhydroxides have been observed in plumes up to 20 km from vent sources, consistent with the above-noted transmissivity anomalies (97). Electron microscopic observations have revealed that oxyhydroxide mineral capsules are associated with cells and show systematic variation as a function of age/distance from plumes. For example, at the Juan de Fuca Ridge, particles with a budding morphology are prevalent in young plumes, while fibrous and star-shaped morphotypes are common in older plume fluids with higher Mn content (95). The morphological forms and proportion of Fe versus Mn oxides vary between plumes as well. For example, twisted stalk morphologies are observed at Axial Volcano but not at other plumes along the Juan de Fuca Ridge (98). Differences in the morphological forms appear to relate to the chemistries of the different vent fields (Axial Volcano versus the Gorda Ridge), in particular the iron and manganese phases (98).

It should be noted that it can be problematic to relate morphological forms of biogenically produced Fe and Mn oxyhydroxide minerals in plumes (or elsewhere, for that matter) to specific bacterial taxa, as there are few representative isolates of Fe- and Mn-oxidizing bacteria for which morphogenic oxide forms can be uniquely associated. As one well-studied example, it is known that *Mariprofundus ferrooxydans*, an Fe-oxidizing proteobacterium, produces morphologically distinct twisted stalks of iron oxyhydroxides during growth (143, 145). While mats of iron oxide particles from Loihi Seamount, from which *M. ferrooxydans* was isolated, are rich in these twisted stalk particles that can be attributed to the *in situ* activity of *M. ferrooxydans*, the mats are also characterized by a variety of other particle morphotypes which currently cannot be associated with specific taxa (144). Hence, often only generalities can be made about the importance of microbes to these particulate-forming processes.

Hydrothermal plume waters are also characterized by elevated concentrations of carbon, which often occur in particulate aggregates of exopolymeric materials, oxides and other minerals, and cells (499). The sticky exopolymeric material that forms the "glue" of these particulate aggregates is exuded by bacteria and phytoplankton. Its production in plumes is hypothesized as one mechanism for trapping redox-sensitive metals used by microbes. Recent investigations using highly resolved X-ray fluorescence mapping documented spatial associations and chemical characterization of Fe(II), Fe(III), and organic carbon in plume particles (557), providing strong support for this hypothesis. Combined with other correlations between iron, organic carbon, and temperature anomalies in plume fluids (43, 499), these studies indicate that plume-associated organic matrices not only trap redox-sensitive metals but also confer distinct chemical properties, such as protection of Fe(II) from abiotic oxidation, which could be advantageous for microbes.

Microbial distribution and diversity. The microbial communities residing in hydrothermal plumes are seeded from high- and low-temperature hydrothermal vent fluids, vent chimneys, and entrained deep seawater. Plumes are invariably associated with elevated populations of microorganisms by comparisons to "background" deep seawater, sometimes containing 10^3 to 10^4 more cells ml^{-1} than ambient seawater (518). Following from the variability in plume chemistry (Table 3), the microbial communities within plumes also vary and reflect these biogeochemical differences, as described below.

The ratio of bacteria to archaea in plume fluids varies considerably. As one example of the relationship of bacteria to archaea, in a plume within the caldera of Suiyo Seamount off Japan, characterized by elevated concentrations of hydrogen sulfide and methane, roughly two-thirds of microbial cells were bacterial, dominated by sulfur-oxidizing *Epsilon-* and *Gammaproteobacteria* (518). In contrast, above the outer rim of the caldera of this seamount, where plume waters are more oxygenated, less than 10% of the total microbial cells were bacterial, indicating an evolving plume microbial population (518). In another study, it was found that archaea comprised 4 to 20% of the microbial communities in plumes above two different vent fields in the Pacific Ocean (536)—the Karei Field at the Central Indian Ridge, characterized by high-tem-

perature, sulfur-rich fluid emission from tall black smoker chimneys, and the Iheya North field at the Okinawa Trough, characterized by cooler, diffuse flow from large (30 to 40 m tall) mounded structures. Archaea in a hydrothermal plume over the Endeavor Segment of the Juan de Fuca Ridge comprised <4% of the prokaryotes there (303). In the available studies, it appears that archaea are less abundant than bacteria within plumes overall, in contrast to the case for the hotter end-member vent fluids from within chimneys (discussed in "Hydrothermal Vents—Vent Fluids and Hydrothermal Chimneys"). However, some evidence demonstrates an increase in the proportion of archaea in ambient oxidized seawater adjacent to a plume (536), suggesting a potential niche for archaea in this transition between the plume and ambient seawater environments.

Within the *Bacteria*, groups of *Gamma-* and *Epsilonproteobacteria* are commonly enriched in plume waters with elevated sulfur concentrations compared to ambient seawater (Fig. 7). Sulfide-oxidizing bacteria make up a large fraction of these groups of proteobacteria (500). For example, in plumes above Karei Field and Iheya North, the *Epsilonproteobacteria* are roughly three times more abundant in the plume waters than in background seawater (536). The uncultivated clades SUP05 of the *Gammaproteobacteria* and SUP01 of the *Epsilonproteobacteria*, thought to represent sulfur oxidizers, comprised almost all of the bacteria in plume waters from the Suiyo Seamount (518). Metagenomic work with the SUP05 clade revealed that these bacteria are chemolithotrophic sulfur oxidizers (584). The same group has been found at Axial Volcano on the Juan de Fuca Ridge (230, 518), at Guaymas Basin (124), and in the Mid-Cayman Ridge (184), suggesting a global distribution.

Gammaproteobacteria also play important roles in the aerobic oxidation of methane, ammonium, and Fe in plumes. The group I and group X methanotrophs of the *Gammaproteobacteria* appear to be the sole methanotrophic members in plumes along the Mid-Atlantic Ridge and Okinawa Trough (141), although further studies are needed to determine how far this pattern extends. Among ammonium oxidizers in plumes, the *Gammaproteobacteria* appear to be an important group (303). Some evidence suggests that lithotrophic *Marinobacter* may be involved in Fe oxidation in plumes (134, 278).

Based on a functional gene survey of cultivated isolates, it appears that the majority of autotrophic *Gammaproteobacteria* in plumes utilize the Calvin-Benson-Bassham (CBB) cycle for carbon fixation, whereas the *Epsilonproteobacteria* utilize the reductive tricarboxylic acid (rTCA) cycle (394). It is suggested that this distinction reflects the positioning of these microbial groups in the plume environment, with rTCA-utilizing autotrophs found in reducing transition zones, such as the interface between fluid or plume waters and seawater (398), taking advantage of the bioenergetic possibilities while protecting oxygen-sensitive enzymes (394). Furthermore, the rTCA cycle requires less ATP and fewer reducing equivalents than the CBB cycle and is therefore energetically more favorable under low-energy conditions such as microaerobic environments (500).

Betaproteobacteria are not dominant in plumes, but ammonia-oxidizing bacteria (AOB) found in the plumes of the Endeavor Segment were predominantly *Nitrosomonas* spp., making up roughly 7% of the total microbial community (302). Phylogenetic investigations indicate that these plume *Betaproteobacteria* form their own clade (303). Likewise, *Deltaproteobacteria* and *Zetaproteobacteria* are also not common in plume waters.

There is some evidence that proximity to the venting source within hydrothermal plumes causes biogeographical patterns in species distribution, which may relate to chemical changes within the plume environment, such as an increasing oxidation state. For example, a cluster analysis of the bacterial 16S rRNA gene clone libraries from Suiyo Seamount revealed that samples less than 2 meters from the vent harbored a different microbial community from that for samples obtained further away (398). Additionally, analysis of 16S rRNA gene clone libraries from the Iheya North plume revealed that the frequency of *Epsilonproteobacteria* decreased with distance from the vent (398).

Compared to the volume of work examining bacteria in hydrothermal plumes, less is known about archaea residing in the plume environment. The proportion of MGI *Crenarchaeota*, which include the ammonium-oxidizing isolate "*Candidatus* Nitrosopumilus maritimus," was found to vary from 2 to 28% of the total prokaryotic community within the first 10 m from the venting source, depending on the vent field and chimney (536). In this study, thermophilic *Euryarchaeota* within the *Methanopyrales* and *Thermococcales* groups dominated near vent sources, while marine group I *Crenarchaeota* organisms increased in percentage with distance from the vent orifice (536). At the Karei vent plume environment, methane- and sulfur-cycling archaea appear to dominate (300). Given the prevalence of archaea in hydrothermal end-member vent fluids and their abundance in the deep ocean (270), future work is necessary to determine the diversity and biogeochemical impact of archaea in hydrothermal plumes.

Similar to the case for archaea, the distribution and function of microbial eukaryotes and viruses in hydrothermal plume environments are poorly known. At the Lucky Strike and Rainbow hydrothermal fields along the Mid-Atlantic Ridge, the presence of ciliates related to *Stylonychia pustulata*, fungi, and alvenellid-related metazoans was detected through 18S rRNA gene surveys (333). In buoyant plume waters close to the vent structures at the Lost City vent field, a clear difference between chimney-associated and water column eukaryotes was observed (335). Dinoflagellates, Euglenozoa, and fungi are abundant in mixed plume fluids and are not found on chimney structures. The group II dinoflagellates, the Syndiniales, are known as parasites of metazoans, and their presence in the Lost City, Lucky Strike, and Rainbow plume fluids indicates that vent fauna may be targets of these parasites.

Viral community dynamics have been evaluated in a few plumes, with the first study conducted at the Gorda Ridge off the northwestern coast of the United States following eruptions in 1996 (266). Although bacterial abundances in those plume waters were elevated at all sampling times (~2, 3, and 11 weeks after eruption), virus/bacterium ratios (<2) were generally lower than those observed in background seawater in the younger plume samples, indicating that the viral abundance in venting fluids may be relatively low (266). These trends were confirmed in a later study of plumes above the East Pacific Rise (615) and in another study of plumes above three vent sites on the Endeavor Segment of the Juan de Fuca

Ridge (430). Later studies of viral communities in hydrothermal plumes indicated a high level of diversity in communities between samples (609, 615) and provided evidence that these viral communities may be largely lysogenic (609), as has been suggested before (363, 593, 610). Furthermore, a survey of viral genetic information suggested that viruses in plume environments have a high degree of genetic novelty compared to those in other environments. Although these initial studies have been informative, understanding the partitioning of microbial eukaryotes and viruses in the plume environment warrants further study before global patterns can be discovered.

MICROBIAL ECOLOGY OF THE DARK OCEAN

Building on roughly 2 decades of individual efforts to identify which microorganisms make up the microbial communities in various habitats of the dark ocean, we have reached a point where broad comparisons can be made among and between environments to identify patterns in microbial biogeography. Questions that can be addressed include the following. Do the same major microbial groups dominate similar habitats, and can patterns be linked to the underlying geochemical conditions of the environments? Are some microbial populations found in many different types of habitats, regardless of the underlying geochemical conditions? Are some types of dark ocean habitats, in general, capable of supporting more microbial diversity than others? If so, do higher diversity levels correlate with the amount of free energy that is available to support metabolic reactions?

One way to address biogeographical patterns is to compare the compositions of phylogenetic libraries constructed from numerous different samples (Fig. 7 and 8), as has been done elsewhere (24; Toner et al., submitted). The 16S rRNA gene is the most widely targeted taxonomic marker in environmental microbiology studies (112) and serves as the most common currency for this comparison. A caveat of such comparisons is that many different approaches are used to generate environmental gene clone libraries—with differences in DNA extraction techniques, PCR primers, and primer annealing temperatures of the PCR being the most important variables—and it is possible that some methods are biased toward amplifying particular microbial groups over others (as an example, see reference 545 for a review of PCR primer bias in amplifying environmental archaeal DNA). While we cannot control for these variations in our analysis, the overarching patterns that are revealed (discussed below) indicate that at the phylum level, such biases may be negligible. Another caveat of such a comparison is that the composition of sequences in an individual clone library may not exactly reflect the composition of microorganisms in the environment, i.e., if 20% of the sequences in a clone library originate from one microbial species, this does not necessarily mean that 20% of the microbes in the samples are that same microbial species. Again, differences between clone library composition and true sample composition can be related to biases in the analytical procedures used; for example, some microbial cells may be lysed easier than others to release their DNA. Additionally, rare species in an environment may be missed if the sequencing depth is not great enough to recover the total diversity in a sample. In most cases, there is a trade-off between cost of sequencing and depth of coverage. A more robust analysis of the true community composition would require analyses such as fluorescence *in situ* hybridization or quantitative PCR with group-specific probes; however, there are far fewer of these data sets available across habitats, and they are usually focused on very specific groups of organisms and not necessarily on the microbial community as a whole.

For the sake of the broad comparison presented here (Fig. 7 and 8), we assume that the clone libraries we considered are roughly representative of the true composition in the environment, given the caveats discussed above. Clone library data sets representing various dark ocean habitats were selected from the literature, focusing on data sets that contained nearly full-length sequences of the 16S rRNA gene (for some habitats with few data available, shorter sequences from fingerprinting analyses such as denaturing gradient gel electrophoresis were also included for completeness) that were generated using domain-level PCR primers (for the *Bacteria* or *Archaea*). Where appropriate, multiple samples from the same site and analysis were pooled (for example, all depths sampled from Ocean Drilling Program [ODP] site 1227 by one researcher were grouped together as one data set, etc.). Over 15,000 selected sequences were aligned against a curated sequence database (using the NAST alignment tool available at http://greengenes.lbl.gov [115]) and manually checked for alignment accuracy against reference sequences from the Greengenes database (116), using the ARB software package (342). For those data sets where only representative clones of an operational taxonomic unit (OTU) were sequenced, the sequence abundance was included in the ARB analysis when it could be deduced from the literature. Further details on methodology are available elsewhere (Toner et al., submitted). For comparison, selected terrestrial and extreme environment habitats, including soils (202), mines (186, 476, 527), microbial mats (469, 475), glacier flows (372), and volcanic and other rock surfaces (215, 293), were also included in the analysis as outgroups. It is important to note that some key dark ocean habitats, including abyssal and trench sediments, have not been well analyzed by domain-level 16S rRNA gene clone libraries.

Broad Patterns in Prokaryotic Microbial Biogeography

The first pattern to emerge from these analyses was the broad (phylum-level) similarity in major microbial groups that dominated similar habitat types (Fig. 7 and 8). In terms of the domain *Bacteria* (Fig. 7), deep marine subsurface sediments are often dominated by either the candidate phylum OP9/JS1 or the phylum *Chloroflexi* (166, 240, 289, 409, 590, 591), although OP9/JS1 organisms are rarely found in other dark ocean habitats and other groups within the *Chloroflexi* are common but not dominant in deep ocean water. In contrast, the *Deltaproteobacteria* comprise a significant fraction of the microbial community in surficial marine sediments, especially those associated with methane seeps (60, 213, 333, 407, 417, 447, 448, 456, 544, 592). The *Gammaproteobacteria* and *Alphaproteobacteria* are the most abundant phyla in the microbial communities harbored by basalts from the seafloor (346, 354, 479). The *Epsilonproteobacteria* appear to dominate most microbial communities associated with active, sulfide-rich hydrothermal chimneys (290, 397, 400, 535, 581); however, inactive

and mantle rock-hosted hydrothermal sulfide chimney material has microbial communities that resemble, at the phylum level, those found on basalts (520, 581; Toner et al., submitted). Hydrothermal fluids, both vent fluids and plume waters, tend to be dominated by either *Gammaproteobacteria* or *Epsilonproteobacteria* (232, 442, 443, 518). The phylum *Acidobacteria*, which is very common in soils (202), is rarely present in dark ocean habitats, with the notable exception of oxic surficial sediments (213, 333, 447, 448, 592). The *Actinobacteria*, which are Gram-positive bacteria with a high G+C content, are also common in surficial sediments (60, 407, 448, 456, 592) as well as in basalt microbial communities (346, 354, 479), but the group is rarely observed in hydrothermal fluids and deep sediments and is not observed in active hydrothermal sulfide chimneys. Members of the phylum *Aquificae* have been observed only in active hydrothermal sulfide chimneys (290, 397, 467). Species from the phylum *Bacteroidetes* (also known as the CFB group) are present in most samples from surficial sediments (60, 407, 448, 544, 566, 592), basalts (346, 354, 479), inactive and ultramafic rock-hosted sulfide chimneys (520, 581; Toner et al., submitted), some hydrothermal fluids, and other dark ocean habitats, such as whale carcasses and sulfidic water columns, but the *Bacteroidetes* are rarely found in deep sediments and active hydrothermal sulfide chimneys. The *Firmicutes* (also known as the low-G+C Gram-positive bacteria) are not observed often in dark ocean habitats, with the notable exceptions of hydrothermal fluids sampled from a sealed borehole on the Juan De Fuca Ridge flank (93, 392) and some basalts and surficial sediments. The *Nitrospirae* are also rarely observed in dark ocean habitats, with occasional appearances in some basalt, surficial sediment, and sulfide chimney communities (407, 479, 520, 535, 592). The phylum *Planctomycetes* appears to have a cosmopolitan distribution, albeit at usually low population levels. The phylum comprising *Thermales* and *Deinococcus* is rarely observed in dark ocean habitats, except in some hydrothermal settings. Cultivated *Thermales* organisms are all thermophilic, and *Deinococcus* organisms are thought to require organic-rich diets, so the absence of these groups in most dark ocean habitats is not surprising. The *Verrucomicrobia* phylum is found in surficial sediments and some hydrothermal fluids but not in deep sediments or active hydrothermal sulfide chimneys.

Similar types of habitat patterns emerge in comparing members of the domain *Archaea* (Fig. 8), as has been observed elsewhere, although with a different naming convention for uncultured archaeal clades (24). Deep marine subsurface sediments (240, 409, 436, 508, 509) are dominated by members of the crenarchaeotal clades DSAG/MBGB and MCG (see reference 545 for more information on naming conventions), which are rarely found in other dark ocean habitats and for which there are no cultivated members. In contrast, archaeal communities in surficial marine sediments at methane cold seeps (195, 213, 417, 592) are often dominated by members of the phylum *Methanomicrobia*. Many hydrothermally influenced habitats have archaeal communities dominated by members of the phyla *Archaeoglobus*, *Thermococcales*, and *Methanococcales* (A/T/M in Fig. 8 and 13) (206, 290, 392, 397, 407, 408, 442, 467, 535, 581). Basalt crust-related samples (135, 163, 232, 354; B. N. Orcutt, K. J. Edwards, and E. Banning, unpublished data) have archaeal communities dominated by members of the MGI clade, which currently has only two cultivated representative genera, "*Candidatus* Nitrosopumilus" and *Cenarchaeam* (200, 288). Other archaeal clades without cultivated representatives, such as the marine benthic groups A, D, and E (MBGA, MBGD, and MBGE), the deep-sea hydrothermal vent euryarchaeotal (DHVE) group, and the marine hydrothermal vent group (MHVG), are also observed occasionally in dark ocean habitats, sometimes as the dominant archaeal phylum (as in the case of DHVE being the most abundant phylum in some sulfide chimney communities [224, 520, 524]).

As stated earlier, the previous analysis assumed that the structure of 16S rRNA gene clone libraries was a reflection of the true structure of the various dark ocean communities considered. However, one must consider that some dark ocean habitats, such as deep sediments, challenge currently available molecular methods and that 16S rRNA gene clone libraries may therefore give a biased view of microbial communities. As one example, recent research (45) that used whole-genome amplification techniques to analyze the community structure of deep sediments from the Peru Margin indicated that phylogenetic inferences vary depending on how the data are analyzed. Studies that examined 16S rRNA genes revealed an apparent dominance of *Crenarchaeota* (47), while analysis of environmental ribosomal proteins indicated that *Euryarchaeota* and *Bacteria* were dominant (488). The difference may be due in part to the fact that fewer representative *Crenarchaeaota* organisms have sequenced genomes, and thus the assignment of proteins to the *Crenarchaeota* is skewed and underestimated.

Taxonomy of Dark Ocean Prokaryotic Microbial Communities

Moving to more selective scales, are there particular genera that commonly occur in the various phyla that are observed in dark ocean habitats? In addition, can metabolic functions be associated with the observed genera? Our analysis, as presented in Table S1 in the supplemental material and in Fig. 9 to 13, reveals that some phyla are represented in many habitats by numerous common genera and families, such as many of the proteobacterial groups and the *Bacteroidetes*, while other phyla have members that are associated mostly with clades of species without cultivated representatives, such as observed for the phyla *Chlorobia*, *Chloroflexi*, *Planctomycetes*, and *Verrucomicrobia*. At the same time, even those phyla represented by classifiable genera also have significant clades of unclassifiable members that are dominated by environmental sequences and no known cultivated members.

For example, one of the most commonly occurring clades of *Alphaproteobacteria* groups within the order *Rhizobiales*, family *Hyphomicrobiaceae*, but this clade is distant from any known cultivated microorganism, so the function(s) of the microbes in this clade is unclear (Fig. 9; see Table S1 in the supplemental material). Another closely occurring clade within the *Hyphomicrobiaceae* is related to the *Pedomicrobium* genus, whose cultivated members are known to occur in biofilms and to oxidize iron and manganese. Cold sediments, basalts, inactive chimneys, and some dark ocean water columns also harbor species within the unclassified OM75 clade (453) that is distantly related to the *Sphingomonadales* order. *Rhodobacteraceae* is a commonly occurring *Alphaproteobacteria* family in

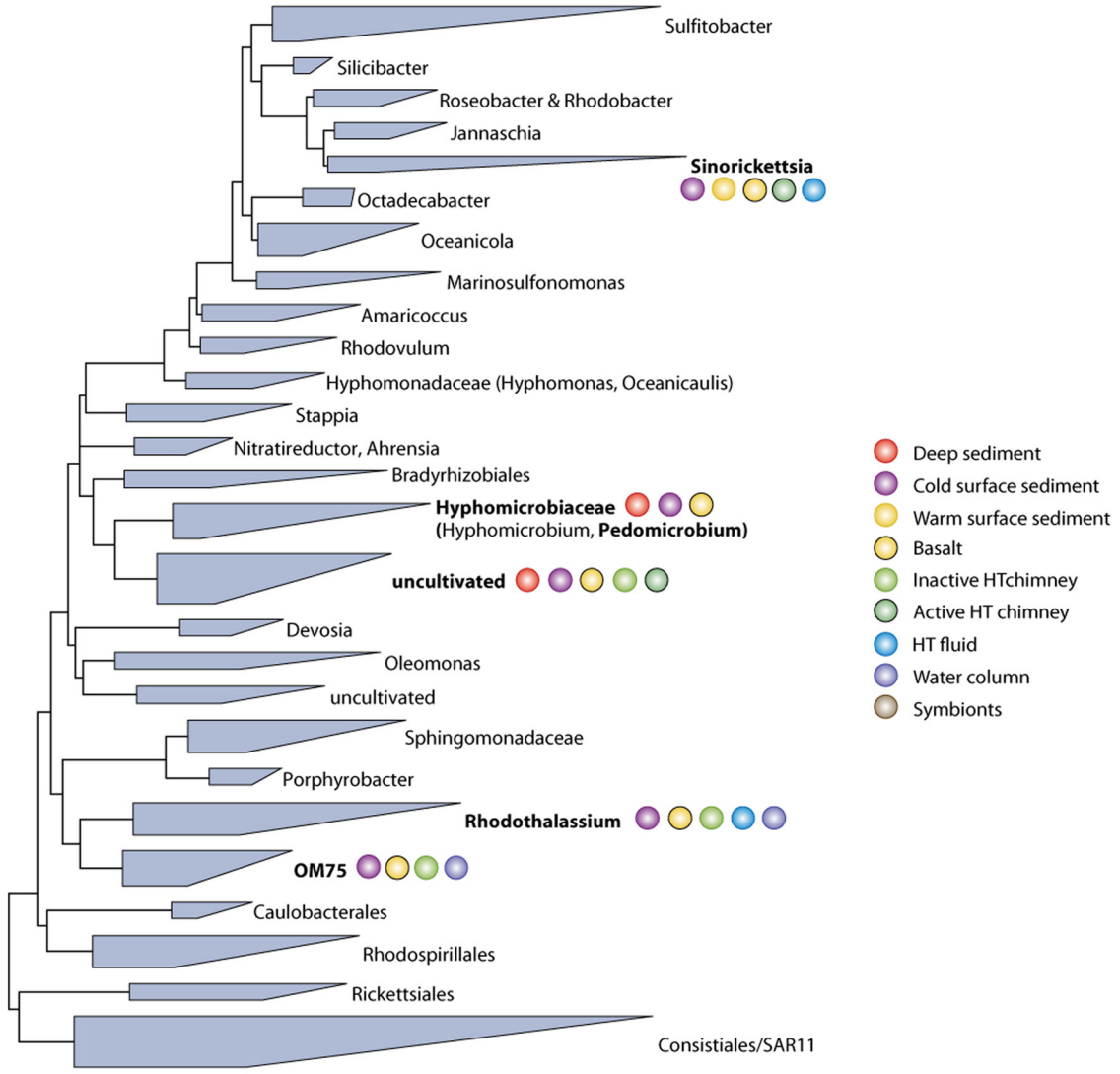

FIG. 9. Phylogenetic tree of *Alphaproteobacteria* groups commonly found in dark ocean habitats. The most common groups are indicated with bold text. Colored symbols indicate which habitats are represented in the common groups.

dark ocean habitats, with environmental sequences most often related to the genera *Jannaschia*, *Marinosulfonomonas*, *Oceanicola*, *Roseobacter*, *Rhodobacter*, and *Rhodothalassium*. A number of dark ocean environmental alphaproteobacterial sequences are also related to the SAR11/*Consistiales* order.

In contrast, most *Betaproteobacteria* from dark ocean habitats are closely related to known genera, mostly found within the order *Burkholderiales*, although they do not appear to be very abundant in most samples. One exception is the genera *Ralstonia*, which does appear with some frequency in samples from hydrothermal fluids (232, 443), basalts (479), and deep sediments (240, 409). This genera is also commonly abundant in the terrestrial deep subsurface (476), and some members of this genera are known as hydrogen-oxidizing, metal-tolerant lithoautotrophs (364).

Common classifiable genera of the *Gammaproteobacteria* include *Acidithiobacillus* (known to metabolize reduced sulfur and iron), *Marinobacter* (known to metabolize hydrocarbons and reduced iron), *Alcanivorax* (whose members are known as halophilic hydrocarbon degraders), *Halomonas* (whose members are organisms known as halophiles capable of denitrification under anaerobic conditions), *Pseudomonas* (whose members commonly include aerobes with a wide metabolic capability), *Thiomicrospira* (known to oxidize sulfur and reduce nitrate), and *Leucothrix* (whose members are known as filamentous chemoheterotrophs capable of thiosulfate oxidation) (Fig. 10; see Table S1 in the supplemental material). Two commonly occuring clades of uncultivated *Thiotrichales* species in the *Piscirickettsiaceae* family (distantly related to the *Thiomicrospira* genus) are dominated by worm symbionts and dark ocean habitat microorganisms. Another large clade with many symbiont and dark ocean habitat species groups with *Thiohalophilus thiocyanoxidans*, a halotolerant chemolithoautotrophic anaerobe that grows with nitrite and thiocyanate (510). Other *Thiotrichales* clades, commonly found in surficial sediments, include the giant sulfur-oxidizing bacteria within the genera

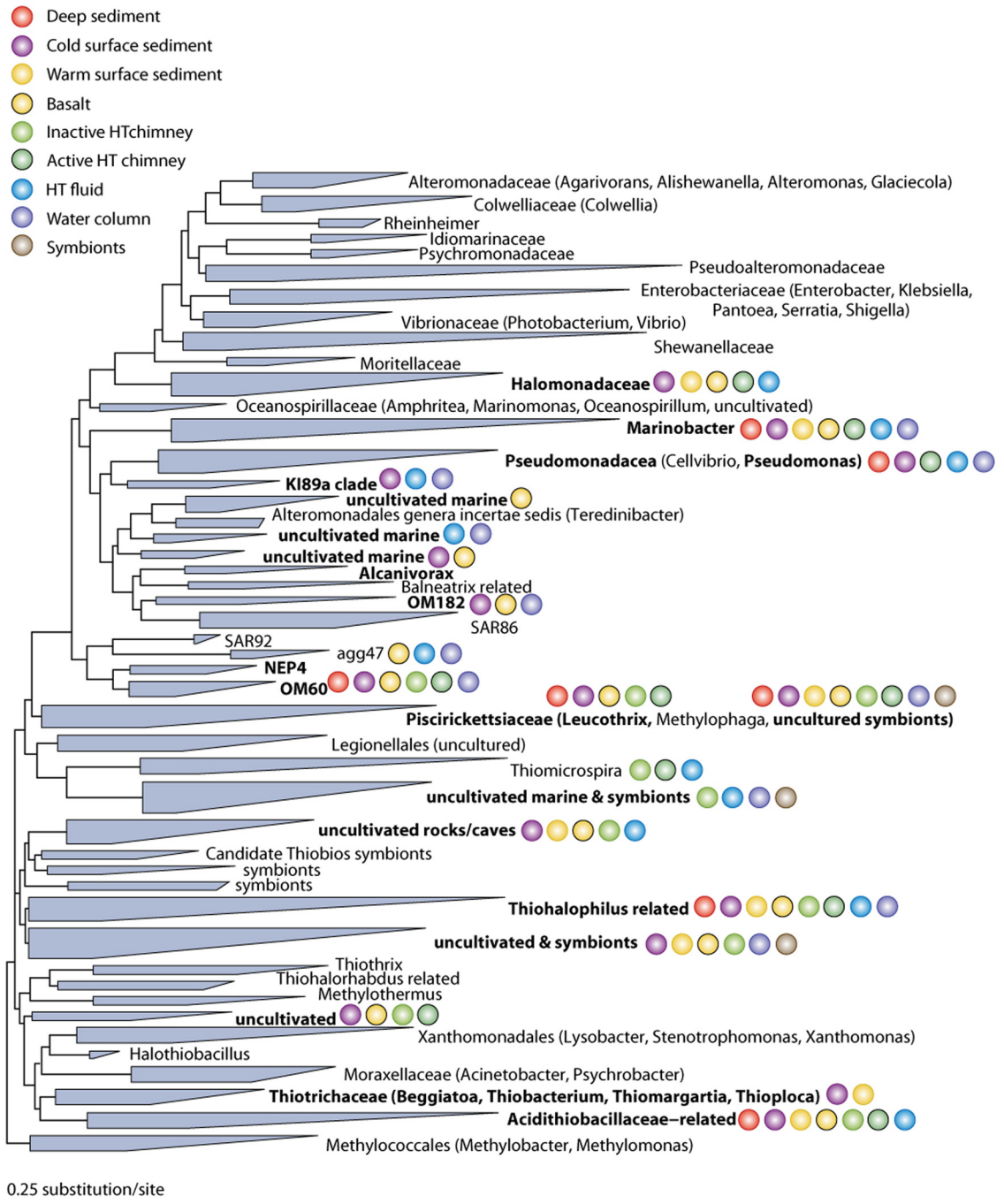

FIG. 10. Phylogenetic tree of *Gammaproteobacteria* groups commonly found in dark ocean habitats. The most common groups are indicated with bold text. Colored symbols indicate which habitats are represented in the common groups.

Beggiatoa, *Thioploca*, *Thiothrix*, *Thiobacterium*, and *Thiomargarita*. Finally, species from fluids and surficial sediments fall within several clades of the unclassified oligotrophic marine gammaproteobacteria (OMG) (85), including the K189A, NEP4, OM60, and OM182 clades; some deep sediments, basalt-related habitats, and inactive chimneys also contain OM60-related species.

Multiple clades of *Deltaproteobacteria* (Fig. 11; see Table S1 in the supplemental material), some closely related to cultivated microorganisms, as well as other clades consisting of only uncultivated members, occur commonly in sediments and other dark ocean habitats. A clade of bacteria closely related to the SAR276 cluster (617) of the *Bdellovibrionaceae* family occurs frequently in sediments, basalt-related habitats, and some sulfide-rich environments. Another common clade found in basalt-related habitats, sulfide chimneys, and some sediments groups with the *Nitrospina* genus of the *Desulfobacterales* order, whose members are known as nitrite oxidizers. Within the

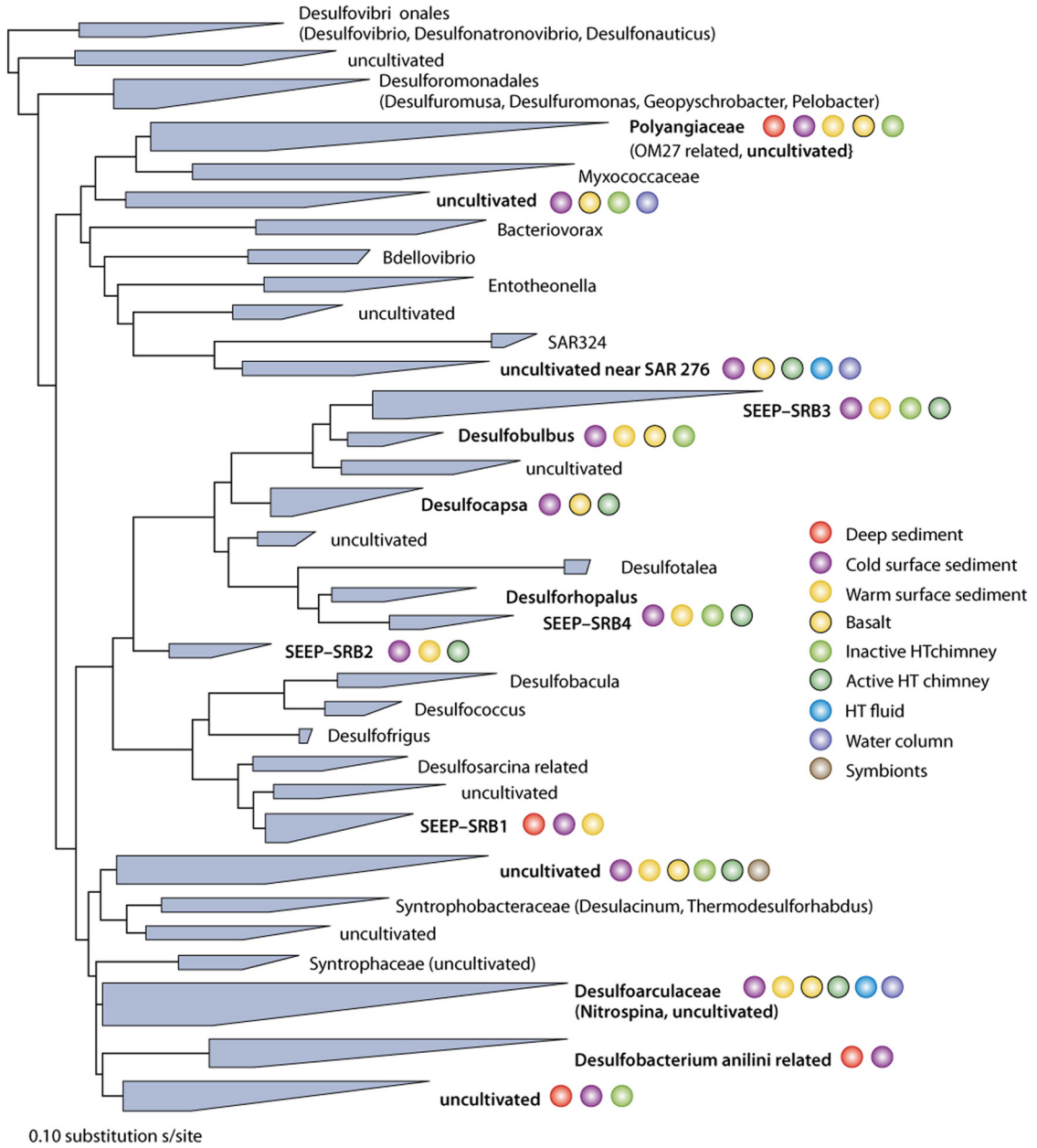

FIG. 11. Phylogenetic tree of *Deltaproteobacteria* groups commonly found in dark ocean habitats. The most common groups are indicated with bold text. Colored symbols indicate which habitats are represented in the common groups.

Desulfobacteraceae family of the *Desulfobacterales* order, clades related to the uncultivated SEEP-SRB1 group (287) and to the genera *Desulfosarcina*, *Desulfococcus*, and *Desulfobacterium* are common in marine sediments. The *Desulfobulbaceae* family is also common in sediments, symbionts, sulfide chimneys, and whale carcass microbial communities. There is also an unclassified clade branching within the *Desulfobacterales* with representatives from deep sediments and some sulfide chimney samples. The *Desulfomonodales* and *Syntrophobacterales* orders occur occasionally in dark ocean habitats, and there is one unclassified clade that groups near the *Syntrophobacterales* that commonly occurs in sediments, basalt-related habitats, and some sulfide chimneys. Additionally, there is another unclassified clade within the *Myxococcales* order that contains members from sediments, basalts, and some sulfides.

Numerous clades of *Epsilonproteobacteria* appear to be important in dark ocean habitats (Fig. 12; see Table S1 in the supplemental material). One often-occurring clade is related to the genus *Sulfurospirillum*, also referred to as subdivision E, within the order *Campylobacterales* (see reference 76 for a recent review of the classification of *Epsilonproteobacteria*). Members of this genus are known to be metabolically versatile, and some are microaerophilic sulfur oxidizers (75). This clade is often found in sediments, sulfide chimneys, whale carcasses, symbionts, and hydrothermal fluids. Another common clade within the *Campylobacterales* is related to the genus *Arcobacter*;

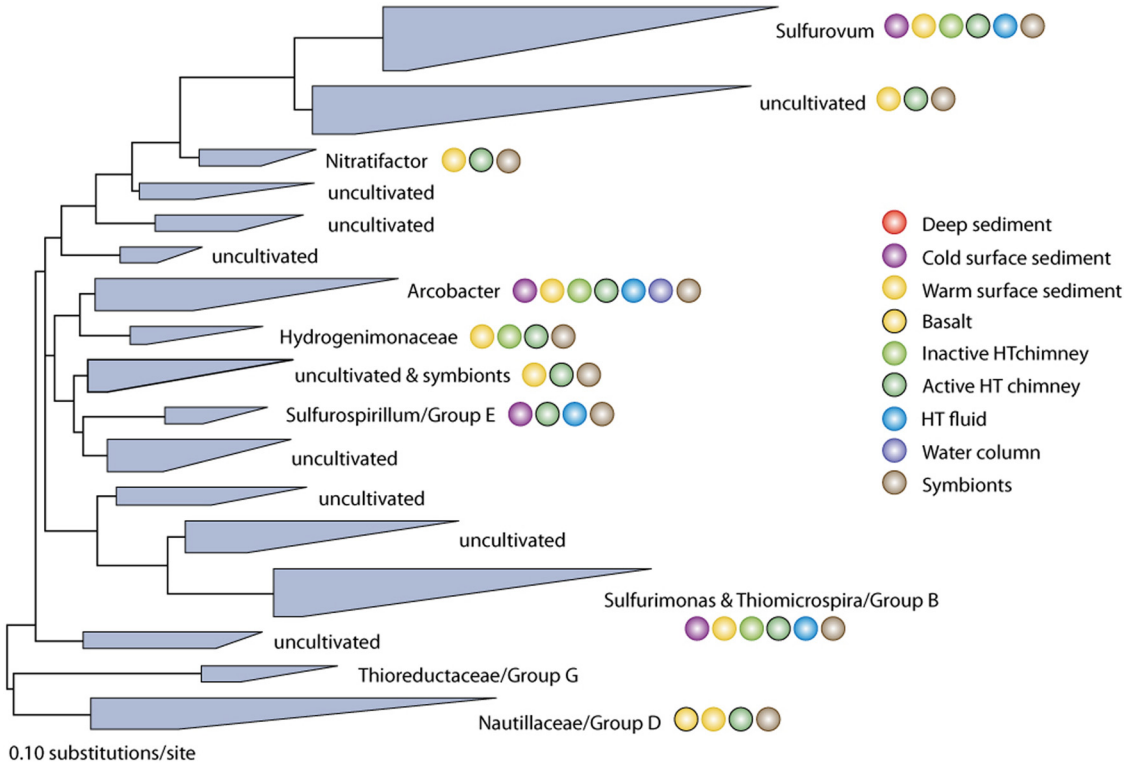

FIG. 12. Phylogenetic tree of *Epsilonproteobacteria* groups commonly found in dark ocean habitats. The most common groups are indicated with bold text. Colored symbols indicate which habitats are represented in the common groups.

Arcobacter-related sequences have been recovered from similar habitats to those of *Sulfurospirillum*. While many cultivated members of the *Arcobacter* genus are known as human and animal pathogens, others appear to be found in environments with opposing gradients of sulfur and oxygen and may be autotrophic. The family *Hydrogenimonaceae*, typified by the species *Hydrogenimonas thermophila*, which was isolated from a sulfide chimney and is known as an autotroph able to oxidize hydrogen by using nitrate, oxygen, or elemental sulfur (532), is often found in active hydrothermal sulfide chimneys, hydrothermal sediments, and hydrothermal plume particles. The genera *Caminibacter* and *Nautilla* of the *Nautiliales* order (also referred to as group D of the *Epsilonproteobacteria*), whose cultured representatives include autotrophs and mixotrophs that couple hydrogen oxidation to S and/or N reduction to produce H_2S and NH_3 (582), are also common in sulfide chimneys, hydrothermal plume particles, basalts, and symbionts. Within the family *Thiovulgaceae*, the *Sulfurovum* and *Nitratifractor* genera, as well as an uncultivated clade, all of which fall within the "group F" subdivision of the *Epsilonproteobacteria*, are found in surficial sediments, symbionts, and hydrothermal habitats. Cultivated members of these genera are known as autotrophic denitrifiers that use sulfur, thiosulfate, and hydrogen as electron donors (244, 399).

Within the *Actinobacteria* (see Table S1 in the supplemental material), the two most commonly occurring clades are found within the *Acidimicrobiales* order, although both clades do not contain cultivated representatives. One of the clades is distantly related to *Microthrix parvicella*, a filamentous heterotrophic bacterium that requires reduced sulfur (50). This clade was recently named ocean crust clade XIII for having members from basalt microbial communities (354), although members of this clade have also been found in sediments and ultramafic rock-hosted chimneys. The other uncultivated clade, which is found in basalt-related habitats, hydrothermal plumes and chimneys, and surficial sediments, is even more distantly related to cultivated species, including the type species *Acidimicrobium ferrooxidans*, the most recognized Fe-oxidizing species from continental acid mine drainage. Other *Actinobacteria* orders are occasionally observed in dark ocean habitats, including one uncultivated clade that is deeply branching within the *Actinobacteria* and is found in deep and surficial sediments.

Families of the *Aquificae* phylum have been found in the dark ocean, predominantly from hydrothermally influenced environments (see Table S1 in the supplemental material). The *Aquificaceae*, *Hydrogenothermaceae*, and *Desulfurobacteriaceae* families of the *Aquificales* are observed principally in hydrothermal deposits and fluids. Additionally, isolated *Aquificae* organisms, including *Thermodesulfobacterium hydrogeniphilum* (251), a heterotrophic sulfate-reducing bacterium, and *Thermodesulfatator indicus* (385), an autotrophic sulfate-reducing bacterium, both of the *Thermodesulfobacteria* class, as well as *Marinitoga hydrogenitolerans* (450), an anaerobic organotroph of the *Thermotogae* class, appear to be environmentally relevant in hydrothermal environments.

Within the *Bacteroidetes*, three clades often observed in dark ocean habitats (see Table S1 in the supplemental material) all

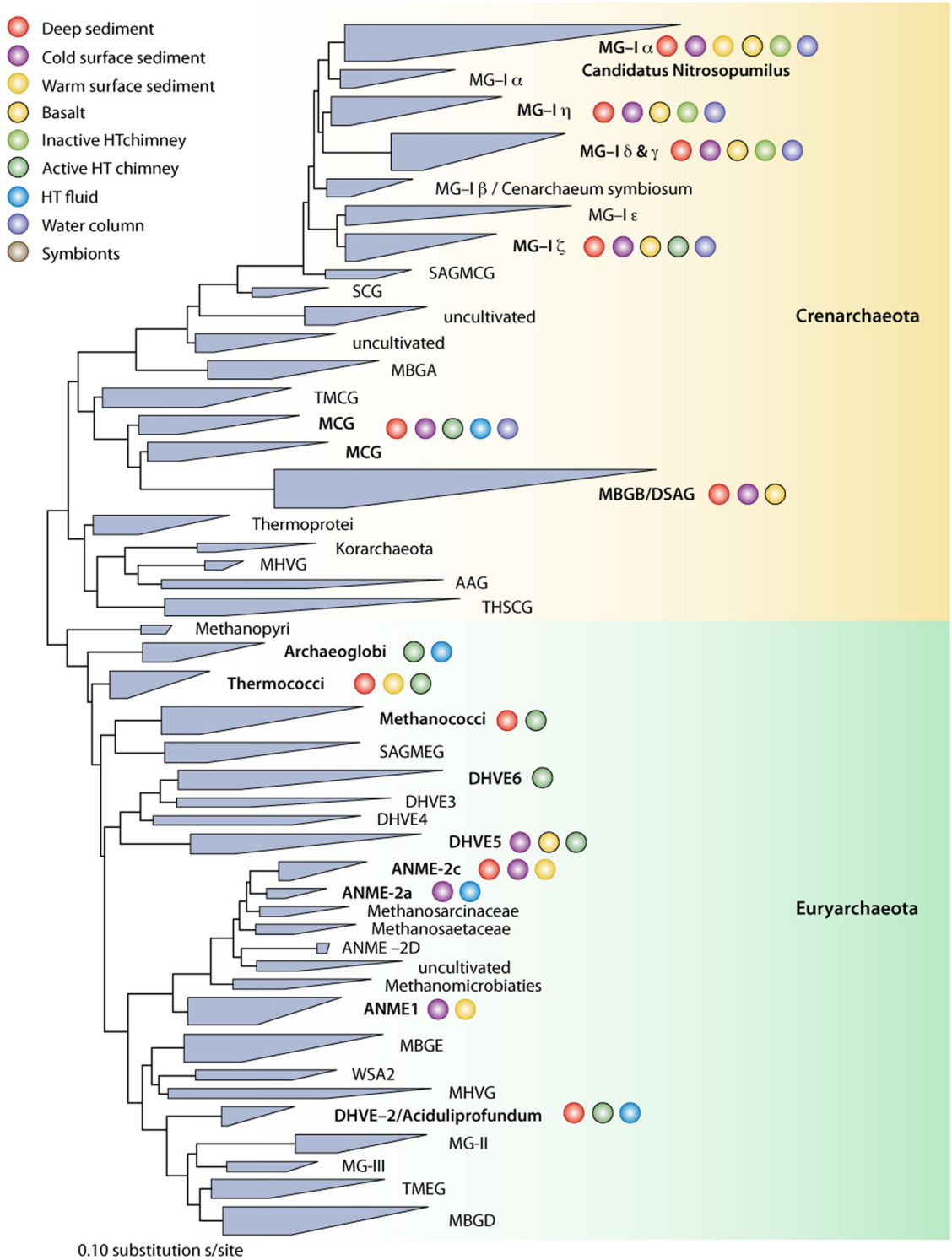

FIG. 13. Phylogenetic tree of *Archaea* groups commonly found in dark ocean habitats. The most common groups are indicated with bold text. Colored symbols indicate which habitats are represented in the common groups.

contain only uncultivated members and group within the *Flavobacteriales* order, which contains metabolically versatile genera, including halophilic, aerobic chemoheterotrophs as well as many pathogens. Other known genera of the *Flavobacteriales* that are occasionally found in dark ocean habitats include *Winogradskyella*, *Flavobacterium*, *Ulvibacter*, *Venteria*, *Polaribacter*, and *Chryseobacterium.* Multiple dark ocean habitats harbor species that are closely related to *Flexibacter tractuosus*

of the *Sphingobacteriales* order. *Flexibacter* bacteria are found in oxic, anoxic, sulfidic, and acidic environments, and they are commonly known as fish pathogens. Within the *Sphingobacteriales*, there is also a clade that is found in multiple dark ocean habitats, and the closest cultivated species is *Cytophaga* sp. strain Dex80-37. Other *Sphingobacteriales* from dark ocean habitats include species related to the genera *Persicobacter*, *Haliscomenobacter*, *Lewinella*, and *Salinibacter*. Finally, there is also a large clade of uncultivated species from deep and surficial sediments that groups at the base of the *Bacteroidetes* class.

Numerous genera of the *Firmicutes* are found occasionally in dark ocean habitats (see Table S1 in the supplemental material). Two independent analyses of hydrothermal fluid and deposits from a sealed borehole on the Juan de Fuca Ridge flank found *Firmicutes* to dominate the bacterial communities (93, 392). These communities had species related to *Vulcanibacillus* of the *Bacilliales* order, whose members are known as anaerobic, heterotrophic nitrate reducers; *Carboxydocella* (whose members are CO-utilizing chemolithoautotrophs [506]) and *Desulfotomaculum* (whose members are sulfate reducers that can also fix nitrogen) of the *Clostridiales* order; and *Ammonifex* organisms of the *Thermoanaerobiales* order, which are known to grow anaerobically by coupling hydrogen oxidation to nitrate reduction (234). *Fusibacter* organisms, anaerobic thiosulfate reducers in the *Clostridiales* order, are also found occasionally in sediments and some hydrothermal environments.

Within the *Planctomycetes* phylum, dark ocean species are found in large clades within the families *Planctomycetaceae* and *Kueneniaceae* as well as in clades that are not closely affiliated with cultivated species (see Table S1 in the supplemental material). Basalt-related and some hydrothermal environments host species related to the genera *Pirellula* and *Planctomyces* of the *Planctomycetaceae*, which are aerobic heterotrophs. Some sediments contain species that group with other uncultivated species within the *Kueneniaceae*, and a few species from basalts and sediments are related to the genus "*Candidatus* Scalindua," whose members are autotrophic anaerobic ammonium oxidizers within this family. Several other clades of uncultivated microorganisms from sediments and basalts group within the *Planctomycetales*.

For those bacterial phyla that occur less often in dark ocean habitats, there are still patterns evident in the genera that are found (see Table S1 in the supplemental material). Although the *Acidobacteria* are not very common in dark ocean habitats, those that have been found in some surficial sediments and in basalt microbial communities mostly appear to be related to the uncultivated group 10 clade (202). Within the *Chlorobia* phylum of green sulfur bacteria, the species found in various dark ocean habitats are associated only with uncultivated clades that do not appear to group within the *Chlorobiales* order. Similarily, the *Chloroflexi* (also known as the green nonsulfur group)-related species that are commonly found in deep sediments group within a clade of uncultivated microorganisms that is distantly related to the *Dehalococcoidetes*, which are known to metabolize halogenated organic compounds. Several surficial sedimentary environments contain microorganisms that are related to the anaerobic fermenters of the *Ilyobacter* genus of the *Fusobacteria* phylum as well as of the *Spirochaeta*. Within the *Nitrospirae*, most dark ocean species from sediments and sulfidic environments are related to "*Candidatus* Magnetobacterium" and *Thermodesulfovibrio*, while basalt-hosted *Nitrospirae* organisms are related to the nitrite oxidizer *Nitrospira marina*. Although species of the *Deinococcus-Thermus* phylum are rarely observed in hydrothermal dark ocean habitats, they tend to be related to the *Oceanithermus* genus of the *Thermales* order, whose members are known as sulfate reducers and microaerophilic chemoheterotrophs. The most common clade of *Verrucomicrobia* in the dark ocean, found in sediments, hydrothermal fluids, basalts, and symbionts, groups with uncultivated species of subdivision 6 of the *Verrucomicrobiales*. The recently described *Zetaproteobacteria* phylum, typified by the type species *Mariprofundus ferrooxydans*, the microaerophilic Fe oxidizer noted above (145), has been documented mostly for hydrothermal environments but also for some sediments.

There are other phylum-level bacterial lineages of uncultivated microorganisms that are found in the dark ocean, although at relatively low frequencies (see Table S1 in the supplemental material). The metabolic strategies of the species in these lineages are unknown. One lineage, marine group A, which is related to the SAR406 cluster, is a common group that is found in many dark ocean habitats, including deep seawater, surficial sediments and basalts, and some hydrothermal fluids. The OP3 lineage, which groups near the *Verrucomicrobia* and *Planctomycetes*, is occasionally found in basalts and sediments. The WS3 candidate phylum has been observed in sediments and basalts. The TM6 phylum, which groups near the *Deferribacteres*, occurs in some basalts, sediments, and hydrothermal environments.

Within the domain *Archaea*, which has far fewer cultured microorganisms, large portions of the dark ocean archaeal communities group within clades of other uncultivated microorganisms (Fig. 8). Some of these clades have been given names based on the environments where they were first found (i.e., deep-sea hydrothermal vent *Euryarchaeota* [DHVE] groups 1 to 7 and marine benthic groups A to E) or even more vague classifiers (i.e., marine group I [MGI] of the *Crenarchaeota*), as summarized previously in a review on archaea from the deep subsurface (545). The addition of even more environmental sequences has caused a ballooning of these descriptive clade names into various subgroups, such as the alpha, gamma, delta, and epsilon subgroups of MGI.

In some dark ocean habitats, the archaeal communities are dominated by microorganisms that group within uncultivated lineages (Fig. 13; see Table S1 in the supplemental material). For example, most deep sediments harbor archaea that group within either MBGB (also known as DSAG), MCG, or the South African gold mine *Euryarchaeota* group (SAGMEG) (Fig. 8). The function of the archaea in these clades is highly speculative. Some reports suggest that some members of the MBGB are heterotrophic methane oxidizers, based on environmental data (for example, see reference 47), while other studies of hypersaline microbial mats hint that the MBGB may be involved in sulfate reduction (469). An exception to this pattern is found in some warmer deep sediments, where members of the *Thermococci* class are also present (473). Although methane cycling is suggested to occur in some deep sediments, there are few published reports of known anaerobic methanotrophs of the *Methanomicrobia* class of *Euryarchaeota* occurring (473). This is in contrast to the case for surficial sediments

from methane seeps, where the *Methanomicrobia* dominate the archaeal communities. Depending on the site, the uncultivated clades of ANME-1, which are distantly related to the *Methanomicrobiales* and *Methanosarcinales* orders, the ANME-2 groups, which are within the *Methanosarcinales*, and/or ANME-3, which groups within the *Methanosarcinaceae* near the *Methanococcoides* genus, dominate the archaeal communities. There are no cultivated representatives of these clades, but all have been attributed to mediating the anaerobic oxidation of methane (53, 417, 429). Members of the ANME clades have also been documented at the Lost City hydrothermal field carbonate chimneys (493), in a hydrothermal chimney from the Logatchev site on the Mid-Atlantic Ridge (581), and in hydrothermal rusts formed from Juan de Fuca Ridge flank boreholes (392). Archaea related to other known *Methanomicrobia* genera, including *Methanococcoides*, *Methanosaeta*, *Methanoculleus*, *Methanogenium*, and *Methanocorpusculum*, also occur occasionally in sediments, particularly those that experience warmer temperatures.

The archaeal communities in warmer sediments tend to exhibit similar dominant groups to those in hydrothermal chimney habitats, including the *Archaeglobi*, *Methanococci*, and *Thermococci* classes and the uncultivated DHVE groups of the *Euryarchaeota*. Within the *Archaeglobi* class, the *Archaeoglobus* genus is found most frequently in hydrothermal habitats, followed by *Ferroglobus*. *Archaeoglobus* organisms are known as thermophilic, heterotrophic sulfate and thiosulfate reducers, while *Ferroglobus* organisms have been shown to anaerobically oxidize Fe and to reduce nitrate and thiosulfate. Hydrothermal environments also support archaea related to the *Methanococcus* genus of the *Methanococci* class, known for producing methane from hydrogen and carbon dioxide at mesophilic to thermophilic temperatures. The *Thermococcus* genus of the *Thermococci* class is often found in hydrothermal habitats and also in some deep sediments. *Thermococcus* species are known to reduce elemental sulfur to hydrogen sulfide while oxidizing organic carbon at thermophilic temperatures. *Pyrococcus* organisms, known as hyperthermophilic anaerobic fermenters, are also observed occasionally at some hydrothermal chimneys and in deep warm sediments. Within the DHVE subgroups, the most common clades appear to be the DHVE-5 and -4 groups, occurring in hydrothermal chimneys, basalts, surficial and deep sediments, and some symbionts. Some hydrothermal chimneys also host species within the *Thermoprotei* class of the *Crenarchaeota*, including the thermophilic to hyperthermophilic *Desulfurococcaceae*, *Pyrodictaceae*, *Thermofilaceae*, and *Thermoproteaceae* families. Occasionally, hydrothermal habitats also contain archaeal communities that cluster within uncultivated clades termed the marine hydrothermal vent group (MHVG) and the terrestrial hot spring *Crenarchaeota* group (THSCG).

In contrast, basalt, cold sediments, and hydrothermal fluid archaeal communities tend to be dominated by members of marine group I of the *Crenarchaeota* (Fig. 8 and 13; see Table S1 in the supplemental material). Recently, an autotrophic, aerobic, ammonium-oxidizing archaeon, "*Candidatus* Nitrosopumilus," became the first cultivated organism to group within the alpha subgroup of MGI. Basalts, deep and surficial sediments, and hydrothermal chimneys all host archaea that group with MGI-alpha, as well as with the delta, gamma, epsilon, eta, and zeta subgroups (subgroup naming by K. Sørensen and A. Teske, unpublished data), which do not currently have cultivated representatives. The other known cultivated organism within MGI (in the beta subgroup) is *Cenarchaeum symbosium*, which was isolated as an endosymbiont of marine sponges and whose genome content also indicates the possibility for chemolithotrophic growth on ammonia (200). This subgroup of MGI does not appear to occur in the dark ocean with much frequency. Recently, it was suggested that the mesophilic MGI group and its relatives should be reclassified as a new phylum entitled *Thaumarchaeota* that is separate from the hyperthermophilic *Thermoprotei* of the *Crenarchaeota* (66).

Cultivated Prokaryotes from the Dark Ocean

In microbial ecology, we consider that fewer than 1% of the species in an environmental microbial community have been cultivated (14, 237). This percentage may be even lower for some dark ocean habitats that have yielded few environmental isolates, such as deep sediments and oceanic crust. Despite the low proportional representation of prokaryotic isolates from dark ocean habitats, the few representatives available are highly valuable for discovering information about unusual types of metabolism, pressure and temperature adaptations, and growth under "extreme" conditions (extreme compared to human-experienced conditions, although not extreme in the sense that such conditions are common on Earth). Biochemical investigations of dark ocean-derived isolates can be rewarding for biotechnology research as well, especially since research on piezophilic and thermophilic microorganisms may lead to the development of new enzymes for pressure- and heat-tolerant applications. For example, lipid-degrading enzymes found in microorganisms that colonize whale falls have been explored for commercial use as low-temperature detergent agents. Here we present and discuss some of the recently cultivated microorganisms from the dark ocean (Table 7). This summary is not meant to be comprehensive but rather to illustrate the breadth of different organisms and lifestyles present in the dark ocean as well as to highlight areas requiring focused culturing efforts in the future.

To begin, a few patterns emerge regarding cultivation yield and proportional representation from the various dark ocean habitats. For example, while the *Crenarchaeota* are numerically dominant in the aphotic pelagic ocean (Fig. 8), they are rarely cultivated. Similarly, the *Alpha-* and *Betaproteobacteria* are common in the aphotic pelagic ocean, yet they are also not commonly cultivated. Cultivated *Euryarchaeota* from the dark ocean often originate from extreme environments such as hydrothermal vents, although this skew may reflect the relatively more intense cultivation efforts at these intriguing locations. Similarly, the *Epsilonproteobacteria* are relatively heavily represented in the isolate list, likely because of their prevalence in sulfidic hydrothermal systems. Gammaproteobacterial species are commonly cultivated from dark ocean habitats, due in part to their positive response to a commonly used cultivation medium, i.e., marine broth 2216 (625). Noticeably absent from our survey (Table 7) are bacterial representatives of the SAR202 group or the *Chloroflexi*, despite the abundance of

TABLE 7. Representative isolates cultivated from the dark ocean[a]

Species	Temp preference (T_{opt} [°C])[b]	Pressure preference (P_{opt} [MPa])	Oxygen tolerance[c]	Carbon source[d]	Energy usage[e]	Location[f]	Habitat[g]	Reference
Euryarchaeota								
Aciduliprofundum boonei	T (70)		AnOx	H	O	EPR, Lau	Chim	465
Geoglobus ahangari	H (88)		AnOx	A	FeR	Guay	Chim	273
Methanocaldococcus indicus	H (85)		AnOx	A	MG	CIR	Chim	318
Methanococcus jannaschii	H (85)		AnOx	A	MG	EPR	Chim	254
Methanococcus strain CS-1	H (85)		AnOx	A	MG	Guay	HTsed	255
Methanopyrus kandleri strain 116	H (122)	40	AnOx	A	MG	CIR	HTfluid	531
Methanothermococcus okinawensis	T (60–65)		AnOx	A	MG	Iheya	Chim	525
Methanotorris formicicus	T (75)		AnOx	A	MG	CIR	Chim	533
Thermococcus barophilus	H (85)	40	AnOx	H	O	MAR	Chim	349
Thermococcus gammatolerans	H (88)		AnOx	H	O	Guay	Chim	253
Thermococcus sp. GY868 and GY869	H (80)		AnOx	H	O	Guay	HTfluid	17
Crenarchaeota								
Aeropyrum camini	H (85)		Ox	H	O	Suiyo	Chim	395
Deltaproteobacteria								
Desulfobulbus mediterraneus	M (25)		AnOx	H	SR	Med	Sed	480
Desulfonauticus submarinus	T (45)		AnOx	H	SR	EPR	Polychaete	23
Gammaproteobacteria								
Halomonas profundus	M (25)		Ox	H	O	MAR, Rainbow	Shrimp	501
Marinobacter alkiliphilus	M (30–35)		AnOx (fac)	H	NTR	Chamorro	Sed	528
Moritella abyssi	P (10)	30	AnOx (fac)	H	O	Atlantic	Sed	620
Moritella japonica DSK1	P (10)	50	AnOx (fac)	H	O	Pacific	Sed	419
Moritella profunda	P (6)	20–24	AnOx (fac)	H	O	Atlantic	Sed	620
Oceanisphaera litoralis	M (28–35)		Ox	H	O	Pacific	Sed	471
Photobacterium profundum	P (10)	10	AnOx (fac)	H	O	Ryukyu	Sed	420
Pseudomonas sp. MT-1	M (30)	0.1	Ox	H	DNTR	Mariana	Sed	537
Psychromonas profunda	P (6)	15–20	AnOx (fac)	H	O	Atlantic	Sed	621
Rheinheimera pacifica	M (4–37)		Ox	H	O	Pacific	Water	472
Shewanella benthica strains DB5501, DB6101, DB6705, and DB6906	P (10)	50	Ox	H	O	Pacific	Sed	276
Shewanella piezotolerans WP3	M (15–20)	20	AnOx (fac)	H	FeR	Pacific	Sed	586
Shewanella psychrophila WP2	P (10–15)	20	AnOx (fac)	H	O	Pacific	Sed	586
Shewanella profunda	M (25–30)	10	AnOx (fac)	H	O	Nankai	Sed	555
Shewanella violacea DSS12	P (8)	50	Ox	H	O	Pacific	Sed	276
Thiomicrospira thermophila	M (35–40)		μOx	M	SOx	TOTO	HTfluid	523
Epsilonproteobacteria[h]								
Caminibacter hydrogeniphilus	T (60)		AnOx	M	HOx	EPR	Polychaete	6
Caminibacter mediatlanticus	T (50)		AnOx	A	DNRA	MAR, Rainbow	Chim	582
Caminibacter profundus	T (55)		μOx or AnOx	A	SR, DNRA	MAR, Rainbow	Chim	377
Hydrogenimonas thermophila	T (55)		μOx or AnOx	A	SR, DNRA	CIR	HTfluid	532
Lebetimonas acidiphila	T (55)		AnOx	A	SR	TOTO	HTfluid	522
Nautilia profundicola	T (41-45)		AnOx	H	SR	MAR	Polychaete	75
Nitratifractor salsuginis	M (37)		μOx	A	NTR	Iheya	Chim	399
Nitratiruptor tergarcus	T (55)		μOx	A	NTR	Iheya	Chim	399
Sulfurospirillum sp. strain Am-N	T (41–45)		AnOx	A	SR	MAR	Polychaete	75
*Sulfurimonas autotrophica**	M (25)		μOx	A	SOx	Hatoma	HTsed	243
*Sulfurovum lithotrophicum**	M (28–30)		μOx	A	SOx	Iheya	Sed	244
*Thioreductor micantisoli**	M (32)		AnOx	A	SR, NTR	Iheya	HTsed	393
Zetaproteobacteria								
Mariprofundus ferrooxydans	M (10–30)		μOx	A	FeOx	Loihi	HTfluid	145
Clostridiales								
Clostridium caminithermale	T (45)		AnOx	H	O	MAR	Chim	65

Deinococcus-Thermus								
Marinithermus hydrothermalis	T (67.5)		Ox	H	O	Suiyo	Chim	477
Oceanithermus profundus	T (60)		μOx	H	O, L (fac)	EPR	Chim	376
Thermus thermophilus Gy1211	T (75)	≤200	Ox	H	O	Guay	HTfluid	348
Vulcanithermus mediatlanticus	T (70)		μOx	H	O, L (fac)	MAR, Rainbow	Chim	378
Thermotogae								
Marinitoga piezophila KA3	T (65)	40	AnOx	H	SR	EPR	Chim	4
Aquificales								
Balnearium lithotrophicum	T (70–75)		AnOx	A	H, Ox	Suiyo	HTfluid	530
Desulfurobacterium atlanticum	T (70–75)		AnOx	A	SR, DNRA	MAR	HTfluid	319
Desulfurobacterium pacificum	T (75)		AnOx	A	SR, DNRA	EPR	HTfluid	319
Persephonella hydrogeniphila	T (70)		AnOx	A	H, Ox	Suiyo	HTfluid	396
Thermovibrio guaymasensis	T (75–80)		AnOx	A	SR, DNRA	Guay	HTfluid	319
Deferribacteres								
Caldithrix abyssi	T (60)		AnOx	H	DNTR	MAR, Logatchev	Chim	375
Deferribacter abyssi strains JR and DR	T (60)		AnOx	A		MAR, Rainbow	HTfluid	379
Deferribacter desulfuricans	T (60–65)		AnOx	H	SR, NTR, AsR	Suiyo	Chim	526
Firmicutes								
Caminicella sporogenes	T (55–60)		AnOx	H	Ferm.	EPR	Polychaete	5
Vulcanibacillus modesticaldus	T (55)		AnOx	H	O	MAR, Rainbow	Chim	317
19 isolates	T (65)		Ox	H	AmmOx	MAR, Snake	Chim, HTfluid	366
Fungi, Basidiomycota								
Cryptococcus surugaensis	M (25)		Ox	H	O	Pacific	Sed	390
Rhodotorula benthica	M (25)		Ox	H	O	Pacific	Polychaete	389
Rhodotorula calyptogenae	M (25)		Ox	H	O	Pacific	Clam	

[a] This list is not meant to be exhaustive but to provide an overview of the types of microorganisms observed. It focuses on work from the past 15 years, with a few exceptions. All temperatures and pressures are those that were identified as optimal conditions.

[b] T_{opt}, temperature optimum; P, psychrophile (<15°C); M, mesophile (15 to 40°C); T, thermophile (45 to 80°C); H, hyperthermophile (>80°C).

[c] Ox, aerobic; μOx, microaerobic; AnOx, anaerobic.

[d] A, autotroph; H, heterotroph; M, mixotroph.

[e] DNRA, denitrification to ammonium; NTR, nitrate reducer; AmmOx, ammonium oxidation to nitrate; HOx, hydrogen oxidation; SOx, sulfide oxidizer; FeR, iron reducer; SR, sulfate reducer; MG, methanogen; AsR, arsenate reducer; O, organotroph; L, lithotroph; fac, facultative.

[f] Source location of inoculum. Pacific, Pacific Ocean; MAR, Mid-Atlantic Ridge; Snake, Snake vent field; Rainbow, Rainbow vent field; Suiyo, Suiyo Seamount; Guay, Guyamas Basin hydrothermal vent field; EPR, East Pacific Rise hydrothermal vents; Loihi, Loihi Seamount hydrothermal vent field; Iheya, Iheya vent field; Hatoma, Hatoma Knoll, Okinawa Trough; TOTO, TOTO vent field; CIR, Central Indian Ridge; Nankai, Nankai Trough, Pacific Ocean; Chamorro, South Chamorro Seamount on Mariana fore arc.

[g] Habitat for source inoculum. Clam, clam symbiont or epibiont; polychaete, polychaete worm symbiont or epibiont; shrimp, shrimp symbiont or epibiont; Sed, sediment; Chim, hydrothermal chimney; HTfluid, hydrothermal fluid; HTsed, hydrothermally influenced sediments; water, water column.

[h] Dark ocean epsilonproteobacteria observed to use the rTCA cycle to fix carbon.

these groups in some dark ocean habitats, indicating that members of these groups are resistant to the cultivation techniques pursued thus far.

Among cultivated archaea from the dark ocean, many originated from hydrothermal systems. *Methanococcus jannaschii*, an autotrophic methanogenic member of the *Euryarchaeota*, is a commonly studied microorganism, as it was one of the first thermophilic archaea isolated from the then newly discovered hydrothermal vents (254). *M. jannaschii* is easily cultivable and has been used as a model in a range of studies, from studies on the effects of pressure to proteomics to thermotolerance studies (55, 327, 432). It was also the first archaeon with a sequenced genome (72), and it has been the target of evolutionary studies due to its basal position in the tree of life. *Methanopyrus kandleri* strain 116 is another notable methanogenic member of the *Euryarchaeota*, due in part to the novel way that it was isolated (531). *M. kandleri* strain 116 was isolated under high pressure (40 MPa) and temperature (122°C, the current highest temperature record for life) from a black smoker hydrothermal vent. *Geoglobus ahangari* is the first known hyperthermophilic archaeon capable of completely oxidizing complex organic matter to carbon dioxide via Fe(III) oxide reduction or fermentation (273). *Aciduliprofundum boonei* represents up to 15% of archaea in hydrothermal systems globally (465). This archaeal species is involved in hydrothermal S and Fe cycling, which indicates that this species activity may have an important impact on global S and Fe budgets.

The recent cultivation of the first low-temperature member of the *Crenarchaeota*, "*Candidatus* Nitrosopumilus maritimus," an ammonia-oxidizing archaeon (288), was an important breakthrough for several reasons. First, the *Crenarchaeota* are known to be dominant organisms in the dark ocean water column (see "The Aphotic Pelagic Ocean"), but only thermophilic lineages had previously been isolated. Second, subsequent work has shown that ammonia-oxidizing archaea similar to the isolated strain are ubiquitous in marine water columns (173, 618) and that they may be more important than the ammonia-oxidizing bacteria in ammonification. Higher affinities for ammonia than those of bacteria are the likely drivers for their dominance in the water column (350).

Among the *Gammaproteobacteria*, several species with adaptations to psychro- to mesophilic temperatures and high pressure have been isolated repeatedly. The genus *Shewanella* contains a few isolates from the dark ocean, including a few *Shewanella benthica* strains and *Shewanella violacea*. The deep-sea *Shewanella* species appear to belong to their own clade (275), which has been investigated to study adaptations to high pressure and the evolution of proteins in deep-sea bacteria and to search for useful compounds for biotechnology. Considering the metabolic flexibility of *Shewanella* (209), this group is a common model in laboratory studies to examine unusual metabolic pathways. For example, *Shewanella* is often a target for biotechnological applications such as sedimentary biobatteries or microbial fuel cells (412). Furthermore, investigations on the potential of *Shewanella* to use "nanowires" in facilitating metabolism under energy-limiting conditions (198) make this a model organism for exploring the potential for marine sediments to contain natural electrical microbial metabolic networks (411). Other pressure-adapted isolates from the *Gammaproteobacteria* that are commonly studied include *Moritella japonica* (419) and *Photobacterium profundum* (420, 576), both of which are psychrophiles. *M. japonica* has been used as a model to examine how cell wall composition—lipid composition in particular—varies with pressure, which revealed that phospholipid fatty acid content decreases with increasing pressure (152). *P. profundum* grows well in marine agar 2216 at low temperature and moderate pressure, making it an easy target for laboratory studies. It has been used as a model in protein expression studies of pressure adaptation at low temperatures (576). *Marinobacter alkaliphilus* is one of the few microbes to be isolated and completely characterized from the deep subsurface biosphere (528). This facultative anaerobe was isolated from 1.5 m below the seafloor of the South Chamorro Seamount in the Mariana Forearc and is capable of growth at a pH as high as 11.4, with an optimum at 9.0, making it one of the most alkaliphilic organisms known. *Marinobacter* appears to be a commonly isolated genus from dark ocean habitats (278). The genome of *Marinobacter aqueolei* indicates that this bacterium is widely adapted to multiple lifestyles, which is likely why this genus is found in diverse dark ocean settings. *Thiomargarita*, a giant sulfur-oxidizing bacterium that falls within the *Gammaproteobacteria*, was first isolated from sediment underlying the Namibian upwelling system (496). This bacterium is an interesting and important discovery for several reasons. First, it is the largest prokaryote currently known, with a cell volume of 200×10^6 μm, with most of this size attributable to giant NO_3^--containing vacuoles that can harbor up to 800 mM NO_3^- for use in sulfide oxidation. Additionally, *Thiomargarita* spp. are also involved in phosphate cycling, and they co-occur in sediments with high phosphorite deposits (497), implicating them in the formation of these phosphorus sinks. Also, the presence of *Thiomargarita*-like fossils has been detected in 600-million-year-old rocks, indicating that these organisms have existed for a very long time (30).

Epsilonproteobacteria are consistently isolated from sulfidic hydrothermal environments, especially from hydrothermal fluids and plumes, yet there are relatively few studies on the metabolic and biochemical features of these isolates. A survey of several different dark ocean *Epsilonproteobacteria* (denoted with asterisks in Table 7) revealed that they all used the rTCA cycle to fix carbon (521). Considering that the rTCA cycle may have been one of the earliest forms of autotrophy to arise on Earth (76), the finding of the rTCA cycle in hydrothermal epsilonproteobacteria provides the tantalizing suggestion that these microorganisms may harbor clues about early life forms.

Mariprofundus ferrooxydans is another hydrothermal isolate, found to dominate in systems with high levels of reduced iron and low sulfide content, such as that found at Loihi Seamount off Hawaii (144, 145). This species is the type species for a new division of proteobacteria—the *Zetaproteobacteria* (145). *M. ferrooxydans*, a microaerophilic neutrophilic autotrophic iron oxidizer, produces Fe- and C-rich twisted stalks as it grows under laboratory settings, similar to other stalk-forming iron oxidizers, such as *Gallionella* species (145). The observation of such iron oxide stalk-forming microorganisms in a range of hydrothermally influenced environments (144), in combination with evidence of iron oxide stalks in ancient sedimentary rocks (324, 468), suggests that these microorganisms may reveal paleobiological clues about early Earth environmental and microbiological conditions.

Outside the *Proteobacteria*, a number of other bacterial phyla contain isolates originating from the dark ocean. Strains of *Thermus thermophilus*—members of the *Deinococcus-Thermus* phylum and related to the biotechnologically invaluable *Thermus aquaticus* strains used to isolate *Taq* polymerases—have been isolated from the deep sea and are used in lab studies to examine the genetics and adaptations to extreme heat (348). Although not a quantitative estimate, a search for *T. thermophilus* in the Web of Science in the spring of 2009 yielded close to 4,000 articles, indicating its usefulness in the basic and applied sciences. Strains of *Aquificales*, a group that lies at the base of the tree of life and close to the last universal common ancestor (319), have been isolated from hydrothermal habitats of the dark ocean, including *Desulfurobacterium* species from midocean ridges and *Thermovibrio guaymasensis* from the Guaymas Basin (319). All of the deep-sea *Aquificales* are chemolithoautotrophs capable of microaerophilic growth with hydrogen and oxygen (319). Considering their basal position on the tree of life, these metabolic observations may offer clues about early life on Earth, but future work is needed to examine such claims. *Deferribacteres* species isolated from hydrothermal vents, such as *Calditrix abyssi* (375) and *Deferribacter* species (379, 526), are not well characterized but are known to be capable of heterotrophic and autotrophic growth based on sulfur, nitrate, and arsenic cycling.

In the past decade, significant efforts have been made to culture microorganisms from seafloor basalts. This has resulted in the isolation of novel, autotrophic Fe-oxidizing *Gammaproteobacteria* (134) and diverse Mn-oxidizing bacteria (541). The isolated Mn-oxidizing bacteria were all *Alpha-* or *Gammaproteobacteria*, as were most of the Mn-oxidizing isolates grown from a Vailulu'u Seamount basalt (517). Interestingly, many of the isolates from the Vailulu'u Seamount were capable of both Fe and Mn oxidation. A diverse collection of aerobic isolates was also recently cultured from various rocks collected on the Mid-Atlantic Ridge, including *Alpha-*, *Beta-*, and *Gammaproteobacteria*, *Actinobacteria*, and *Firmicutes* (455).

There has been substantial effort put into isolation of deep sedimentary microbes following the realization that deep sediments harbor prokaryotic cells. The first deep subsurface bacterium isolated was *Desulfovibrio profundus*, a sulfate-reducing bacterium isolated from 500 mbsf in the Japan Sea (33). Methanogens have been isolated from methane hydrate-bearing sediments (371). The greatest culturing effort of deep subsurface sediments to date was undertaken during deep drilling in the equatorial Pacific during leg 201 of the Ocean Drilling Program (119, 542). One recent study recovered a remarkable number and diverse population of bacteria from many drill cores during that expedition, including *Alpha-*, *Gamma-*, and *Deltaproteobacteria* and members of the *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* (38). The genera isolated most frequently in that study were *Bacillus* (68 isolates) and *Rhizobium* (40 isolates). Two thermophilic sulfate-reducing bacteria within the genus *Thermosediminibacter* were isolated from multiple cores on the Peru Margin (310), and members of the *Alpha-*, *Delta-*, and *Gammaproteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* were also isolated from the same cruise (119). Isolates that are recovered from drill cores are often viewed with some skepticism because of concerns about contamination and the likelihood that it is actually possible to cultivate *in situ* microbes from deep sediments because of the low growth rates and unknown or cryptic physiological requirements that they may have. Not surprisingly, there are few other cases of substantial recovery of deep sedimentary biosphere isolates (e.g., see references 34, 242, and 556), and those that have been recovered often are not well represented in cultivation-independent surveys of environmental samples (47, 240, 241, 488). Regardless, since little is currently known about microbial life in deeply buried marine sediments, future efforts to culture these microorganisms are likely to provide us with valuable insights into life in the deep subsurface biosphere as well as to potentially provide useful proteins for biotechnology.

Although there are relatively few isolates from the dark ocean in comparison to the total pool of microbial diversity, there have been significant efforts to sequence genomes of the available dark ocean isolates, considering their metabolic and lifestyle novelty, which may yield new genetic information that is useful for biotechnology and for understanding microbial functioning. Completed genomes for isolates in Table 7, as of the time of this writing, are for *M. janasschii*, *Methanopyrus kandleri* (strain AV19), *S. benthica* (strain KT99), *S. violacea* (being sequenced by Keio University and Kinki University/Japan Manufacturing Science Technology Center), *Shewanella piezotolerans*, *P. profundum*, *Nautilia profundicola*, and *T. thermophilus* (strains HB27 and HB8, isolated from hot springs, not the deep ocean [but the same species]). Also completed are the genomes of *Sulfurovum* sp. NBC37-1 and *Nitratiruptor* sp. SB155-2, both isolated from hydrothermal environments but not yet characterized (399). Genomes in progress for isolates from Table 7 that can be searched for gene content but are not yet annotated are for *Thermococcus barophilus*, *Moritella* sp. PE36 and *Psychromonas* sp. CNPT3, both isolated from the deep ocean but not yet characterized, and *Caminibacter mediatlanticus*, *M. ferrooxydans*, "*Candidatus* Nitrosopumilus maritimus," and *Marinitoga piezophila*. This is a total of 18 genomes, including those for both *T. thermophilus* strains, providing enough data to begin to probe the gene set necessary for life in the dark ocean, and perhaps even to pinpoint genes specific to different environments, such as hydrothermal vents versus the aphotic pelagic ocean.

Isolation and culture of dark ocean microorganisms have revealed much about life in the dark at great pressures, extremes of temperature, and gradients of electron donors and acceptors. There are clearly a few lineages where little is known, and these should be targets for future work. Among these are anaerobic methanotrophs, which have been kept alive in their original sediment for study along with their sulfate-reducing bacterial counterparts (189, 402) but never isolated in pure culture. Similarly, microorganisms from deep subsurface oceanic crust have eluded cultivation.

A goal for future efforts should be well-planned laboratory studies using those organisms currently in culture. Some isolates are cultivated and then not fully characterized (e.g., see references 46 and 134), and it is likely that much more can be learned simply by working with already available cultures in addition to isolating those that are underrepresented. Many dark ocean microorganisms have interesting lifestyles and can serve as genetic models for specific areas of research. While rare, a few models have been exploited for their physiological

traits. As mentioned above, *Photobacterium profundum* strain SS9 is used as a model for studying adaptation to life at high pressure (48, 139, 576). It is amenable to transposon mutagenesis (307) and generation of mutants through deletions (140). *Thermus thermophilus* is another genetic model from the dark ocean. This thermophile has been used extensively to determine the structure of the ribosome and for modeling the ribosome's interactions with molecules (71, 306, 611, 622). *T. thermophilus* has also been used to study the structure and function of other cellular components, including respiratory complex I (483), elongation factor P (201), and heme-copper oxidases (190). The two model organisms mentioned here indicate only the tiniest fraction of the potential avenues for study offered by dark ocean microbes. We hope that interested readers may expand on this modest collection of model microorganisms. For example, considering how vast the high-pressure environments of the dark ocean are, further models for high-pressure adaptations should be targets for future efforts.

Microbial Eukaryotes and Viruses in the Dark Ocean

While we are beginning to be able to identify patterns in prokaryotic microbial ecology in the dark ocean, understanding of viral as well as microbial eukaryotic ecologies in the dark ocean is still in its infancy. Although viruses can have a strong influence on shaping the population biology and ecology of microbial communities, both by impacting mortality and by serving as vectors for horizontal gene transfer, little is known about their role in dark ocean habitats. Recent investigations suggest that an average of 80% of global heterotrophic prokaryotic production in the deep sea is recycled due to viral lysis, and this may explain relatively rapid prokaryotic turnover in surficial sediments in nutrient-poor deep-sea ecosystems (104). In some diffuse hydrothermal fluids, it has been shown (609) that the prokaryotic community contains a significant fraction of lysogenic hosts containing temperate viruses (bacteriophages whose genomes incorporate into and replicate with that of the host bacterium, which represents a quasistable relationship until such time when the bacteriophage turns lytic). Additionally, bacteriophages appear to be a less diverse subset of the viral community, with a high incidence (~50%) of novel viral genes that have not been seen in other environments, although different vent samples appear to contain distinct prophage communities (609). In contrast, background deep seawater does not contain as many lysogenic virus-host relationships. These observations support the theory (196) that lysogenic virus-host interactions may be a mechanism for prokaryotes to cope with the challenging physical and chemical gradients experienced in hydrothermal environments, although the mechanisms through which temperate viruses influence phenotype adaptation is uncertain. Whether the degree of lysogenic virus-host interactions correlates with the productivity of an environment or the relative proportion of autotrophy to heterotrophy needs to be determined.

A few studies have been conducted to assess the diversity of microbial eukaryotes in the dark ocean. Alveolata, protozoa of the Chromalveolata kingdom that include ciliates, apicomplexans, and dinoflagellates, appear to be the most diverse lineage in the dark ocean, and novel alveolate clades (marine alveolate groups I and II/Syndiniales) appear to be common in marine habitats (129, 333–335). Hydrothermal sediments appear to contain a diverse assemblage of microbial eukaryotes, including Euglenozoa, Acantharae, Alveolates, Apusozoa, Nematoda, Polychaeta, and Stramenopiles, as well as some novel groups (129, 333). Bodonids and ciliates dominated the eukaryotic community in colonization experiments placed on hydrothermal sulfide chimneys, suggesting that these protists are early colonizers (333). On hydrothermal carbonates from the Lost City hydrothermal field, there is a higher level of diversity of metazoa (Porifera, Cnidaria, Nematoda, and Polychaeta), although the community appears to be dominated by ciliates and Euglenozoa, similar to the sulfide chimney colonizers (335). Alveolates, fungi (particularly a clade of Basidiomycota from hydrothermal and anaerobic environments), and metazoans (including Cnidaria and Copepoda) are detected in the water column above hydrothermal vents (333, 335). In deep polar waters, alveolates are the most diverse planktonic microbial eukaryotes, in particular the marine alveolate groups I and II related to dinoflagellates (334). Yeast groups appear to be the dominant form of fungi found in the deep water column of the dark ocean, although the diversity of genotypes found is relatively low (37). The main groups detected were the Ustaliginomycetes class (smut fungi that cause plant diseases) of the Basidiomycota phylum and the Pezizomycotina of the Ascomycota phylum. Yeasts and fungi have also been described for metal oxide microbial mats and basalts from Vailulu'u Seamount and may play an important role in metal cycling in that environment (90).

MAGNITUDE OF PROKARYOTIC BIOMASS IN THE DARK OCEAN

For those interested in understanding the cycling of carbon and other elements on Earth, it is necessary to quantify the biomass of microbial life in the dark ocean, as this biomass could be a large and dynamic stock of carbon, since the dark ocean comprises the largest habitat on Earth. Unlike terrestrial and upper ocean habitats, which can be visualized easily by satellite imagery and for which corresponding interpretations of biomass can be validated, the dark ocean remains relatively difficult to access, and generalizations and extrapolations must be made from episodic, generally sparse sampling of dark ocean habitats. Back in the 1850s, it was thought that no life existed below 300 fathoms, according to the "azoic hypothesis" of Edward Forbes (165). Following the use of deeper nets and trawls during the HMS *Challenger*, U.S.S. *Albatross*, and other expeditions of the late 1800s, this idea was overthrown; however, it would still take many decades for a better understanding of life in the dark to come about. Our knowledge of the dark ocean has advanced significantly in the last few decades, especially following the discoveries of hydrothermal vents, cold seeps, and whale fall communities in the late 1970s and 1980s (91, 249, 439, 504).

Some of the first evidence for microbial life in deep sediments arose from methanogenic activity studies conducted by Oremland and colleagues on samples that originated from Deep Sea Drilling Project Leg 64 in the early 1980s (428). The first visualization that confirmed the presence of microorganisms in deep marine sediments was published less than 2 decades ago, when Parkes and colleagues demonstrated that mi-

crobial cells exist down to 518 m below the seafloor at five different Pacific Ocean sites and that their decreasing abundance with depth shows a consistent trend (433). This depth-dependent trend of microbial abundance in the deep marine subsurface was the cornerstone of a revolutionary paper by Whitman and colleagues (608) that estimated that a majority of microbial life on Earth is located in the marine subsurface. Whitman et al. estimated that 3.6×10^{30} cells (of a total of $\sim 5 \times 10^{30}$ microbial cells on Earth) are contained in the marine subsurface, which corresponds to 300 Pg of carbon in deep marine subsurface cells, or an estimate of one-third of the carbon on Earth and 50 to 80% of Earth's microbial biomass occurring in the deep marine subsurface. Parkes et al. (433) estimated a somewhat smaller biomass of marine subsurface carbon: ~50 Pg C, or roughly one-tenth of the carbon on Earth. In the intervening 15 years, subsequent studies of more deep marine sediments revealed more variation in the depth-dependent trend of microbial abundance in sediment, with much lower abundances at depth in sediments with very low organic carbon, leading to a significantly smaller (by an order of magnitude) estimate of microbial biomass in the global marine subsurface of 5×10^{29} cells (446), or 5 to 15% of Earth's microbial biomass. Additionally, evidence indicates that the global majority of microbial life in deep sediments is comprised of the *Archaea* (323). Intriguingly, recent analysis suggests that the pattern of microbial abundance and, to some degree, activity in sediments may correlate with Milankovitch frequency variations in paleo-oceanographic conditions, as high cell abundances correlate with organic-rich diatom oozes (3).

Although most attention in quantifying microbial life in the dark ocean has focused on subsurface sediments, the ocean crust is another dark ocean habitat that we currently know even less about. Calculations based on the oxidative state of hydrothermal ridge flank crusts suggest that $\sim 1 \times 10^{12}$ g C year^{-1} of chemolithoautotrophic primary production from Fe and S oxidation and hydrogen production can be supported in these habitats globally (26). This production rate is on the same order as that predicted for subsurface sediments, suggesting that a significant microbial population may also exist in the ocean crust. Finally, the dark ocean water column is estimated to contain 6.5×10^{28} prokaryotic cells (608) and 4×10^{30} viruses (519). Clearly, given the size of the various dark ocean habitats and the potential stocks of biomass carbon harbored within them, further work is necessary to better constrain their relative contributions to elemental cycling on Earth.

CONCLUSIONS AND FUTURE DIRECTIONS

Gradients that govern microbial communities in the dark ocean exist at spatial scales from micrometers to kilometers and temporal scales that range from milliseconds to millenia. In recent years, significant advancements have been made in the study of chemical flow at scales relevant to microorganisms, for example, in using ^{13}C labeling to study carbon flow (56, 187, 341, 451). Similar advancements are just beginning to be applied in the dark ocean today (107, 429), leading to new insights into the relationships and dynamics of the system. However, the vastness of size and the decoupling of biogeochemical processes and cycles—both spatially and temporally—present significant challenges to scientific advancement in microbiological research in dark ocean realms. While microorganisms in the dark ocean are able to react quickly to sudden organic matter inputs, such as whale falls or oil spills, the majority of dark ocean microbial communities live off organic matter created days (in the deep water column) to millennia (in deep sediments) prior to its utilization, and it can be difficult to unravel these extreme scales in our observations and experiments.

Some aspects of microbial life in the dark ocean still offer many mysteries with unanswered questions. What are the sources of energy, electron acceptors and donors, and pools of C, N, and P, and how are they transformed? How diverse and productive are primary producers in the dark ocean? What is the fate of carbon produced by dark ocean microorganisms? By what processes and at what rates are carbon and electrons exchanged between the oceanic crust and the mantle, and what magnitude of influence does microbial life have on these processes? How are the mass cycles and energy flows of geological and biological systems in the deep ocean and subseafloor related? What are the spatial dimensions of dark environments and their interactions with the subseafloor? What are the limits to microbial life? What factors control the redox state of Earth's surface? How do microbial reactions in the dark ocean influence that world in a way relevant to human society (i.e., influence on carbon sequestration, climate change, or industrial applications)? Solving these rich and compelling scientific problems requires integrated and multidisciplinary approaches. Microbiologists are poised to play leading and exciting roles in these endeavors.

Of critical importance is the continued development of a more detailed understanding of the linkages between geochemistry and microbiology. New methods, for example, techniques which allow measurement of *in situ* electrochemical gradients (e.g., see references 191 and 345) in multiple dark ocean habitats, are needed to reach this goal. Improvements are also needed to determine the composition and activity of microbial communities thriving in these biogeochemical gradients. The scale at which measurements are made will vary significantly between different environments and is dependent on the scale of the redox gradients and rates of process, i.e., fine-scale redox measurements at the scale of microorganisms are critical for understanding a compressed redox environment, but they may not be necessary for all systems. Other critical developments that will lead to novel discoveries about life in the dark ocean include the usage of long-term observatories and autonomous and cabled sensors. For example, the development of new autonomous underwater vehicles for "sniffing out" the signals of hydrothermal plumes has enabled the detection of new hydrothermal environments in the world's oceans (184, 326), and underwater sensor packages are revealing new dynamics about microbial activities in the dark ocean (191, 587). Instrumented boreholes drilled into the seafloor are uncovering remarkable findings about how fluids and microorganisms move through the oceanic crust (41, 93, 159, 425, 602). Such instrumented boreholes are long-term (i.e., years to decades) observatories that allow temporal and spatial patterns to be discovered and new hypotheses to be tested about the connectivity and activity of life in the subsurface. Developing and testing new instrumentation for observatory science

in the dark ocean are similar in many ways to planetary and space science programs developing rovers to explore other planets.

We hope that this review has provided an interdisciplinary synthesis of data covering the distribution and activity of microbial life inhabiting the dark ocean. The previous years and decades of exploratory, hypothesis-generating analyses have facilitated an understanding of the relevant patterns and driving factors to explain dark ocean microbiology. As an example, broad patterns of microbial biogeography in the dark ocean emerged from the assemblage of a plethora of 16S rRNA gene surveys from multiple habitats, and they appear to correlate with habitat type. Only with data that recently became available is it actually possible to show, for example, that upper water column microbial communities are distinct from the predominant deep sedimentary communities—which has been expected but not previously demonstrated. The insights gained from increasing and hitherto unavailable information open opportunities to support long-held, untested hypotheses in microbiology, such as the concept of "everything everywhere but the environment selects" (25), or to prove such concepts incorrect. Furthermore, novel intriguing hypotheses and questions have emerged from the newly accumulated data (505); for example, "what is the nature and function of the rare biosphere?" Is the rare biosphere a seed population that persists everywhere, waiting for the right conditions to grow, or does it play a more active functional role that varies from habitat to habitat? We now have enough data, for example, to examine the rare biosphere in hydrothermal systems in separate ocean basins against the backdrop of what is common to both in terms of major players. Many other examples abound and are presented as opportunities for new scientific research, made possible in part by the availability of what is now a fairly large collection of phylogenetic "stamps" from different environments. The combination of this baseline phylogenetic information with sophisticated new *in situ* analyses and experimentation lends itself to novel hypothesis-driven research in the dark ocean, which will undoubtedly lead to new discoveries about how life functions on planet Earth, and possibly beyond.

Dark ocean microbiology research operates at the very limits of existing techniques and at the boundary of our understanding of life on Earth, which is both challenging and stimulating for advancement. Recent technological advances in DNA sequencing and single-cell study are opening up new and exciting pathways for studying microbial life in the dark ocean. The recent revolution in next-generation sequencing technology enables an improved understanding of the rare biosphere in nature (63, 233, 505) and allows comparisons of metagenomic data from geographically and geochemically distinct dark ocean habitats to determine differences in metabolic and life strategy potential (45, 619). As sequencing costs decrease, the possibility of generating nearly complete genomes of uncultivated species from environmental samples is on the horizon, which may reveal new metabolic pathways in dark ocean habitats. Advancement in single-cell analysis—from isolating single cells from environmental samples for genomic amplification (51, 513, 616) to analyzing the isotopic composition of single cells with secondary ion mass spectrometry (107, 388, 583)—will undoubtedly uncover new information linking function and form in environmentally relevant microbial species. To understand how life in the dark ocean survives and thrives requires a truly multidisciplinary and cooperative approach, including scientists from fields as diverse as geology, chemistry, physical oceanography, and engineering, as well as microbiologists, molecular biologists, ecologists, and bioinformaticists. The growing communication between these different fields, coupled with increasingly sophisticated and elegant methods for research, hints at a bright future for understanding microbial life in the dark ocean.

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Beth N. Orcutt received her Ph.D. in Marine Sciences from the University of Georgia in 2007. After conducting 2.5 years of postdoctoral research at the University of Southern California, she became a postdoctoral researcher at the Center for Geomicrobiology at Aarhus University, Denmark, in 2009, where she continues to study microbial life in deep sediments and oceanic crust. She is currently involved in developing new instrumentation and methodologies for exploring life in the hard-to-access dark ocean.

Jason B. Sylvan started off his career studying biogeochemistry in the upper ocean water column. After completing his Ph.D. in Biological Oceanography at Rutgers University in 2008, he dove into dark ocean research as a postdoctoral researcher at the University of Southern California, where he is currently based. Jason studies the biogeochemistry and microbiology of hydrothermal vent plumes, seafloor-exposed basalts and inactive sulfides, and the oceanic crust, and he plans on being a rock star when he grows up.

Nina J. Knab studied molecular biology and zoology at the University of Heidelberg (Germany) before she turned to marine biogeochemistry and obtained a Ph.D. at the Max Planck Institute for Marine Microbiology in Bremen, studying anaerobic oxidation of methane and sulfate reduction in marine sediments. Her interest in early evolution and metabolic energy generation of subsurface microorganisms led her to the University of Southern California, where she is currently working as a postdoc with Katrina Edwards on the potential role of iron oxidation in microbial energy generation in the oceanic crust. Her research aims to combine geochemical and biological constraints on evolution and to understand the limits and extent of life in remote habitats.

Katrina J. Edwards received her M.S. in Geochemistry from the University of Wisconsin-Madison in 1996 and her Ph.D. in Geomicrobiology in 1999, also from the University of Wisconsin-Madison. She joined the faculty in the Department of Marine Chemistry and Geochemistry in June 1999 as an Assistant and then as an Associate Scientist (2006). In 2006, she joined the faculty in the Department of Biology, Marine Environmental Biology Division, where she is now a Full Professor (since 2009). Dr. Edwards is the Director of a newly established NSF Science and Technology Center, C-DEBI (Center for Dark Energy Biosphere Investigations). Her research focuses on the microbiology and ecology of the subsurface biosphere in oceanic ridge flanks, chemoautotrophic iron metabolism, and bioalteration of the ocean crust and hydrothermal sulfides.