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Andreas Teske
Verena Carvalho *Editors*

Marine Hydrocarbon Seeps

Microbiology and Biogeochemistry of a
Global Marine Habitat

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Editors

Marine Hydrocarbon Seeps

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of a Global Marine Habitat

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Foreword

Hydrocarbon seeps are unlike other seafloor ecosystems. Their dependence on fossil carbon sources, strongly reducing redox state, abundant supply of electron donors (hydrogen, sulfide, methane, ammonia), and frequent brine admixture sets them apart. In this book, aficionados of the oily deep provide an overview on hydrocarbon seeps in major marine regions before directing the spotlight onto some of the special microorganisms thriving in these ecosystems. The book opens with an overview on seep microbial ecology in Chap. 1 by S. Emil Ruff, inspired by his publication “Global dispersion and local diversification of the methane seep microbiome,” published in 2015 in *Proceedings of the National Academy of Sciences of the United States of America*. He and his colleagues established the microbial characteristics of seep ecosystems and delineated their differences to other seafloor microbiota. No other benthic microbial ecosystem is so strongly shaped by methane- and sulfur-cycling bacteria and archaea.

Among the bacteria that thrive in hydrocarbon seeps, perhaps none are as diversified and adaptable as the hydrocarbon-degrading sulfate reducers, introduced, and discussed in depth in Chap. 2 by Sara Kleindienst and Katrin Knittel. Taken together, the wide substrate spectrum and environmental tolerance of this group turn these bacteria into effective and ubiquitous catalysts for hydrocarbon oxidation in anaerobic marine sediments.

In the next five Chaps. (3, 4, 5, 6 and 7), the editors of this book and their colleagues, including Samantha B. Joye, Gunter Wegener, Cassandre S. Lazar, and Konstantinos Ar. Kormas draw inspiration from multiple research cruises and introduce hydrocarbon seeps with contrasting characteristics in different geographical regions to highlight some of the inherent variability that make seep habitats and their microbiota such rewarding targets for microbiological and biogeochemical studies. This gallery of seeps opens with introducing a classic site in the Gulf of California, Guaymas Basin, in Chap. 3—an unusual hybrid location that combines characteristics of a mid-ocean ridge hydrothermal vent site and a hydrocarbon seep. Here, sedimentary organic matter in thick sediments covering an active spreading center is transformed into hydrocarbons under high temperature and pressure. The hydrothermally heated mixture of very young petroleum and

hydrothermal energy sources migrates to the sediment surface where it sustains unusually complex microbial communities characterized by extensive thermal tolerance. This chapter also doubles as an introduction to recent advances in alkane utilization and oxidation by novel types of archaea that have been cultured from its hydrothermal sediments, or, that were detected by metagenomic analysis. In contrast to this hydrothermal system, Chap. 4 introduces the most extensive archipelago of cold hydrocarbon seeps on Earth, extending from Florida to Texas along the entire continental slope of the northern Gulf of Mexico. Here, numerous sites represent a wide spectrum of mud volcanoes, seafloor brine lakes, methane hydrate outcrops, and oil seeps that—with few exceptions—remain in the early stages of microbial and biogeochemical surveys and invite further in-depth studies in the future. The widespread hydrocarbon-oxidizing and -assimilating microbial populations in the Gulf of Mexico seeps are of particular environmental significance as they serve as seed banks for hydrocarbon degraders that play a role in natural hydrocarbon remediation, for example, after the Deepwater Horizon oil spill in 2010. A highly unusual seep type in the southern Gulf of Mexico, the Chapopote Asphalt volcano, is singled out in Chap. 5. Instead of gas and liquid hydrocarbon seepage, this site is dominated by the seafloor emergence of slowly flowing, heavily viscous, and chemically weathered asphalt that yet contains sufficient energy sources to sustain its own hydrocarbon seep ecosystem. The habitat surveys are completed by Chaps. 6 and 7 on Mediterranean hydrocarbon seeps, mud volcanoes, and hydrocarbon-rich brine flows, which are embedded in the complex tectonic setting of the Mediterranean Sea, from where they derive some of their distinct qualities.

High concentrations of sulfide, intermixed in the cocktail of reduced gases at seep sites, select for different types of sulfur-oxidizing bacteria, including the highly conspicuous filamentous mat-forming members of the family *Beggiatoaceae*. The filaments are often large enough to be visible to the unaided eye and form extensive white, yellow, and orange-colored mats on the seafloor that coincide with areas of active seepage. At present, these striking bacteria are mostly uncultured; however, the *Beggiatoaceae* are intensively investigated in the context of their habitat. For example, their preferred biogeochemical niche at the sediment–seawater interface is tackled through microprofiler surveys, and physiological experiments are conducted with live mat material. Here, two chapters are devoted to them: In Chap., 8, the two editors of this book summarize and extend current knowledge about the diversity of *Beggiatoaceae* in Gulf of Mexico seeps, while chapter 9, led by Dirk de Beer, opens up a new and previously neglected perspective on the importance of pH and DIC speciation for autotrophic metabolism in *Beggiatoaceae*, using samples collected in Guaymas Basin.

The book concludes with Chap. 10 by Tony Gutierrez and Sara Kleindienst, introducing Stable Isotope Probing (SIP) as a promising and versatile tool to investigate the activities and substrate ranges of hydrocarbon-oxidizing bacteria. SIP elegantly integrates knowledge about the genomic potential of hydrocarbon-oxidizing microorganisms with trophic responses detected in complex mixtures or communities to hydrocarbon availability, using microbial enrichment experiments amended with specific substrates.

Throughout these chapters, all authors have included numerous illustrations and photographs of marine seep ecosystems, keeping in mind that submersible rides and ROV deployments are in short supply, and that many readers have not seen these distinctive seafloor landscapes and microbial habitats in person. Often, images say more than a thousand words; they provide a mental reference and serve as an anchor for the increasingly data-rich studies that are emerging in the productive field of hydrocarbon seep microbiology. An informative and amply illustrated book assists in making the field more accessible because it lowers the “activation energy” barriers that stand in the way of encouraging further interest in deep-sea hydrocarbon seep research. Likewise, this book provides readily accessible background and context for rapidly evolving research “hot spots,” for example, the ongoing discoveries of enzymatic pathways of hydrocarbon degradation, the investigation of genomes and metagenomes, the identification of novel hydrocarbon-oxidizing bacteria and archaea, and new insights into stable carbon isotope systematics and molecular structures of metabolites that arise from new findings in the repertoire of microbial degradation of hydrocarbons. For now, this volume provides a useful introduction, anticipating to stimulate further research interest in hydrocarbon seep microbiology and biogeochemistry.

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Chapter 1

Microbial Communities and Metabolisms at Hydrocarbon Seeps



S. Emil Ruff

Abstract Hydrocarbon seeps are common features of all oceans and are located mainly along the continental margins (Fig. 1). Seeps are locally restricted, yet highly productive hotspots of biodiversity that experience very different environmental conditions and energy regimes than the surrounding deep-sea sediments. Hydrocarbon seep ecosystems are mostly fueled by methane. Occasionally, seeps are found that emit the short-chain hydrocarbons ethane, propane or butane, and even oil and asphalt seeps have been described. Seep ecosystems therefore comprise ecological niches and microbial clades that are distinct from those found in deep-sea sediments, which are not fuelled by methane and other hydrocarbons. This chapter provides an overview of the communities thriving at marine hydrocarbon seeps and the microbial metabolisms that create these oases of life (with references to other chapters in this book). It highlights the current knowledge of the diversity and biogeography of seep microbial communities and presents possible mechanisms governing their community assembly.

Life at Hydrocarbon Seeps

Due to the abundant energy supply from subsurface sources of hydrocarbons, cold seeps sustain microbial and faunal communities with population sizes that can exceed those found in the surrounding marine sediments by several orders of magnitude. A teaspoon of seep sediment may contain tens of billions of microbial cells (10^{10} cells ml^{-1} ; Ruff et al. 2013; Marlow et al. 2014), and a square meter of surface sediment can be covered by tens of thousands of individual animals, such as ampharetid polychaetes (Sommer et al. 2010). The microbial communities of seep sediments belong to the biomass-richest in nature, with cell numbers comparable to those found in soil (Portillo et al. 2013) and the human gut (Sender et al. 2016). This suggests an efficient conversion of available energy sources, but also the existence of

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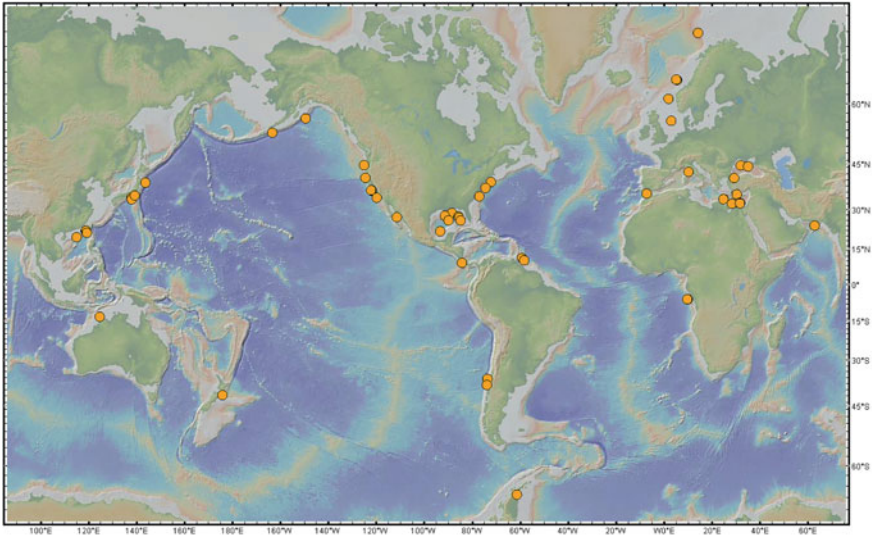


Fig. 1 Map of seafloor regions with investigated marine hydrocarbon seepage (orange dots). The map shows a selection of well-known seep areas. The total number of seeps worldwide is unknown, but estimated to be at least several ten thousand. The map was created using GeoMapApp

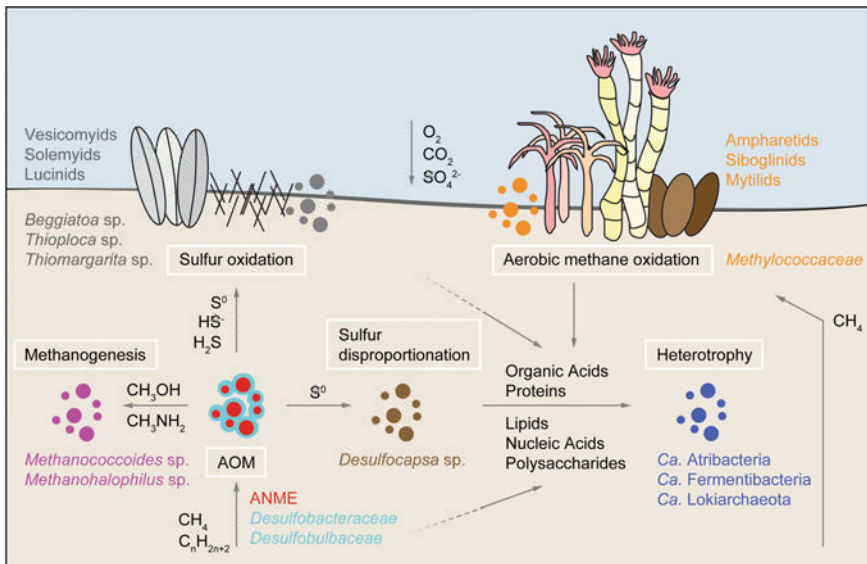


Fig. 2 Schematic overview of the food web and the microbial metabolisms found at hydrocarbon seeps. Methane and hydrocarbon oxidizers are at the base of the food web turning subsurface-derived energy into biomass. Their metabolic activity creates ecological niches for other microorganisms by producing methylated compounds used by methanogens, and sulfur compounds used by sulfur oxidizers and sulfur disproportionators. The organic matter build up by methane- or sulfur-cycling microbes is then used by heterotrophs, further increasing the diversity and complexity of the ecosystem. Note: The species that are associated with a metabolism serve as examples, e.g. *Beggiatoa* sp. are common sulfur oxidizers, and do not represent a comprehensive list

a complex food web with numerous community functions (Fig. 2). At the center of this food web are chemosynthetic microorganisms. These organisms are the primary producers of biomass and catalyze vital ecosystem functions such as aerobic and anaerobic methanotrophy, sulfate reduction, and sulfide oxidation (see, for example, organisms presented in Chaps. 2, 5, and 9). Chemosynthetic microorganisms harness the energy of reduced chemical compounds like hydrogen, sulfide and methane. The microbes use this energy to fix inorganic carbon and synthesize organic carbon compounds and cellular building blocks such as proteins, sugars, lipids, and nucleic acids. The organic carbon produced by chemosynthetic populations is then used by heterotrophic microorganisms or animals in the food web (Fig. 2). Chemosynthesis is performed by free-living microorganisms in the sediment or by host-associated microorganisms living in symbiosis with animals, including mussels, clams, and tube worms (Figs. 2 and 3). The most well-known animal hosts at seeps are siboglinid tubeworms, bathymodiolin mussels, and vesicomylid clams (Fig. 3), which live in

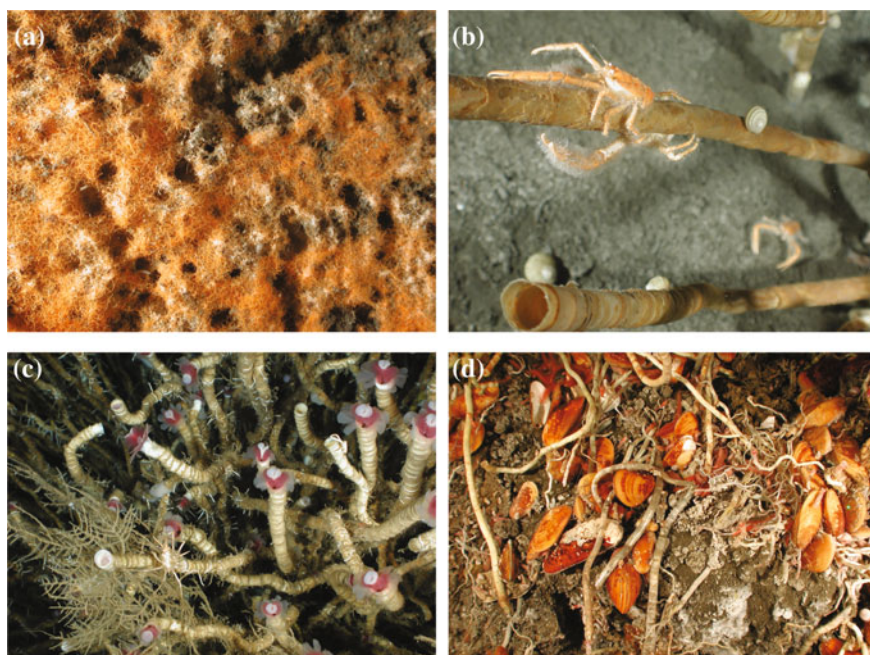


Fig. 3 Seep-associated microbiota and macrofauna. **a** Orange mats are composed of giant filamentous bacteria that oxidize sulfide at the sediment-water interface; **b** Deep-sea crabs are often covered with white filaments of sulfur-oxidizing bacteria growing on their carapace; **c** *Lamellibrachia* tubeworms within the family *Siboglinidae* grow in dense patches and can live for centuries. They lack a mouth and gut, and are dependent on nutrition provided by sulfur-oxidizing endosymbionts. **d** Mytilid mussels of the genus *Bathymodiolus* live on the sediment surface and harbor sulfur- and methane-oxidizing endosymbionts. (Images courtesy of I.R. MacDonald, Florida State University)

symbiosis with methane-oxidizing or sulfur-oxidizing bacteria (Cordes et al. 2005; Levin 2005; Decker et al. 2013).

Life Styles at Hydrocarbon Seeps

Aerobic Methanotrophy

Aerobic methanotrophic bacteria use oxygen to consume methane and are found in the upper, mostly oxic sediment layers of hydrocarbon seeps (Yan et al. 2006; Lösekann et al. 2007; Tavormina et al. 2008; Wasmund et al. 2009). At seeps with a high fluid flow this oxic layer is only a few millimeters thick. Sediment surfaces may become completely anoxic when the seafloor is covered by microbial mats of sulfur-oxidizing bacteria that consume the oxygen before it penetrates into the sediment (Fig. 3a). More details about mat-forming sulfide-oxidizing bacteria are presented in Chaps. 8 and 9. With decreasing fluid flow and increasing bioirrigation by macrofauna, the thickness of the oxic layer can increase to several centimeters. Aerobic methanotrophs often occur in sediments that are not covered by microbial mats, and are bioirrigated by tubeworms (Sommer et al. 2010; Ruff et al. 2013; Thurber et al. 2013), or in sediments that are frequently disturbed, such as the active centers of mud volcanoes (Niemann et al. 2006; Felden et al. 2010, 2013). Aerobic methanotrophs are generally less abundant in seeps with low and stable fluid fluxes, e.g., above methane hydrates (Ruff et al. 2015). However, the occurrence of aerobic methanotrophic *Methylococcales* in hypoxic and even anoxic/sulfidic layers at hydrocarbon seeps indicates that these organisms can also adapt to high-sulfide and low-oxygen conditions (Lösekann et al. 2007; Pachiadaki et al. 2010; Roalkvam et al. 2011; Ruff et al. 2013). Some aerobic methanotrophs are even active in anoxic waters of the Black Sea (Blumenberg et al. 2007), present in oxygen minimum zones of the Pacific Ocean (Hayashi et al. 2007), and in anoxic peat soils (Roslev and King 1994), surviving under prolonged anoxic conditions (Roslev and King 1995).

Seep ecosystems seem to harbor mainly type-I methanotrophs of the order *Methylococcales*, which cluster among at least three distinct phylogenetic groups (Fig. 4 red clades) (Inagaki et al. 2004b; Yan et al. 2006; Lösekann et al. 2007; Tavormina et al. 2008; Wasmund et al. 2009; Foucher et al. 2010; Ruff et al. 2013, 2015; Thurber et al. 2013; Felden et al. 2014; Oshkin et al. 2014; Paul et al. 2017). The aerobic oxidation of methane with oxygen is one of the most energetically favorable reactions in the microbial realm, yielding up to 800 kJ per mol methane oxidized. The type-I methanotrophs use the ribulose monophosphate (RuMP) pathway, a highly efficient metabolism for fixing carbon (Kato et al. 2006), which likely provides an advantage at seep systems. Hence, these aerobic methanotrophs are relatively fast growing, and are among the early colonizers of seep ecosystems (Ruff et al. 2019). The use of alternative electron acceptors, such as nitrate, or novel pathways to perform methane oxidation in anoxic habitats, has been indicated in the order *Methylococcales* (Costa et al. 2017; Martinez-Cruz et al. 2017), which may explain the occurrence of



Fig. 4 16S rRNA gene based phylogenetic tree showing common seep-associated bacteria, color-coded according to their energy metabolism: red = aerobic methane oxidizers, blue = sulfur oxidizers, brown = sulfate reducers, black = heterotrophs. Scale bar: 10% estimated sequence divergence

active aerobic methanotrophs in anoxic methane seep sediments (Ruff et al. 2013). At seep ecosystems, aerobic methanotrophs are also common as symbionts of mussels (Duperron et al. 2008, 2011) and siboglinids (Dubilier et al. 2008; Hilário et al. 2011) (Fig. 2). The animals shelter the microorganisms in specialized organs or tissues, providing them with oxygen and methane. In return, the microorganisms provide reduced organic carbon compounds for their hosts.

Anaerobic Methanotrophy Coupled to Sulfate Reduction

Anaerobic methanotrophic archaea (ANME) dominate anoxic sulfidic sediments and typically occur in consortia with sulfate-reducing bacteria (SRB) (Fig. 5). These consortia perform the anaerobic oxidation of methane (AOM) coupled with

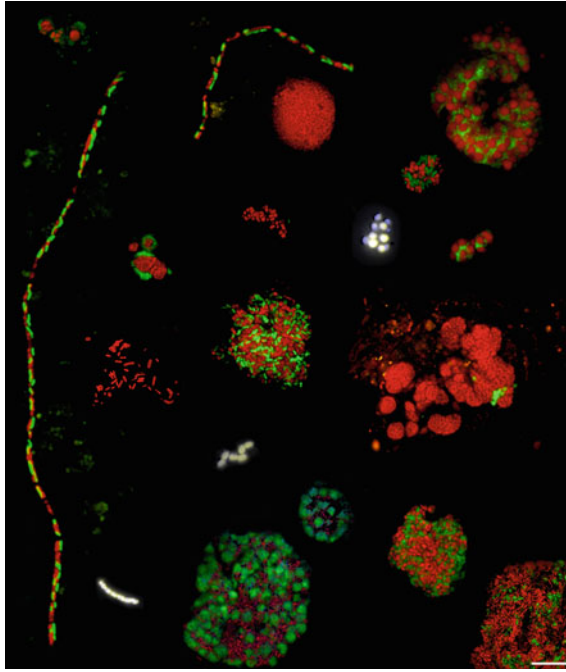


Fig. 5 Compilation of fluorescence micrographs of anaerobic methanotrophic archaea (red = ANME) and sulfate-reducing bacteria (green = SRB), which play key roles in the anaerobic oxidation of methane in marine sediments. The organisms can occur by themselves, but are mostly found together, forming syntrophic consortia. Aerobic methanotrophic *Methylococcales* (beige) do not need a partner to metabolize methane. Close relatives of free-living *Methylococcales* are found as chemosynthetic symbionts of marine invertebrates (Petersen and Dubilier 2009). Together, aerobic and anaerobic methanotrophs remove around 30% of the methane emitted from the seafloor of the world's oceans, and hence greatly impact the global methane budget (Boetius and Wenzhöfer 2013). Scale bar = 20 μm . *Figure credit* Katrin Knittel, S. Emil Ruff

sulfate reduction (SR) (Knittel and Boetius 2009). AOM in Guaymas Basin sediments is presented in Chap. 3.

The AOM net reaction



yields between -20 and -40 kJ per mol methane in situ, and AOM is thus one of the least favorable energy-conserving catabolic reactions in nature. The produced carbonate often precipitates on the seafloor, forming chemohierms that are used as substrate for macrofauna. The produced sulfide is an energy-rich compound that diffuses upwards creating niches for sulfide-oxidizing bacteria at the sediment surface (for more details refer to Chaps. 8 and 9).

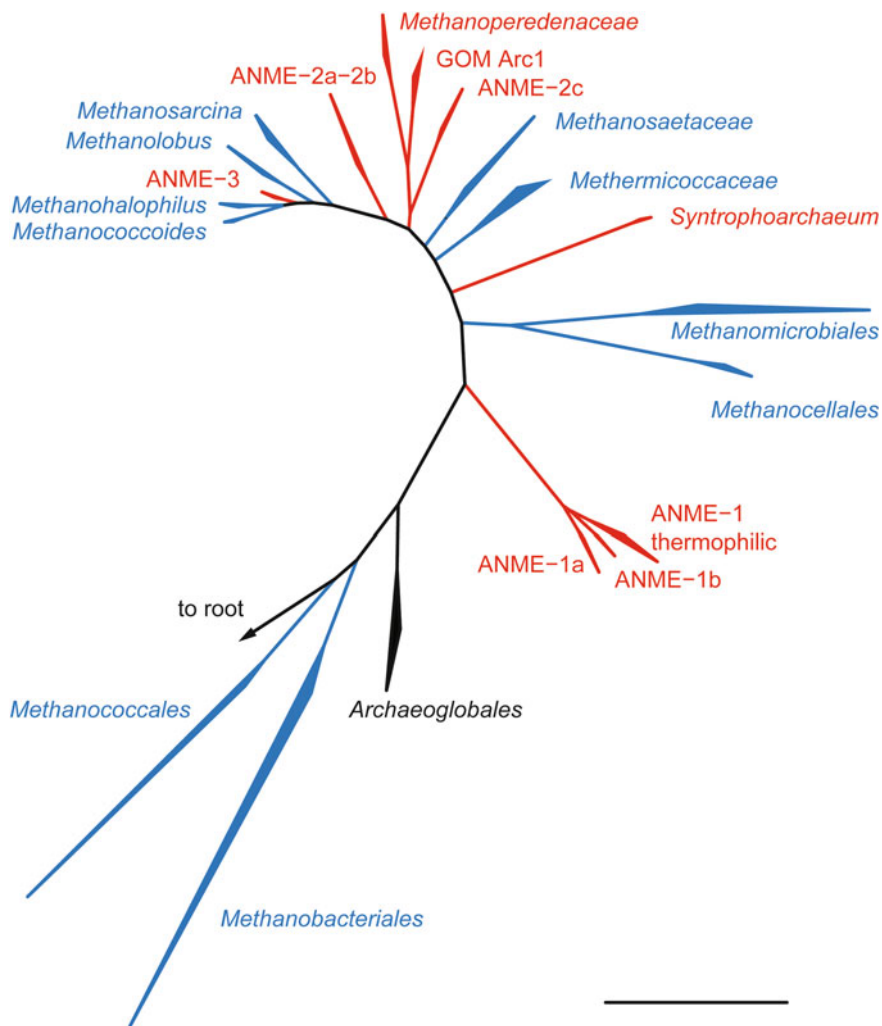


Fig. 6 16S rRNA gene based phylogeny of ANME archaea showing the affiliation of anaerobic hydrocarbon-oxidizing archaea (red) in relation to their methanogenic relatives (blue) within the class *Methanomicrobia*. Scale bar: 10% estimated sequence divergence

ANME belong to the class *Methanomicrobia* and affiliate with three major clades: ANME-1, ANME-2 and ANME-3 (Hinrichs et al. 1999; Orphan et al. 2002; Niemann et al. 2006) (Fig. 6). These major clades comprise the subgroups ANME-1a, ANME-1b, thermophilic ANME-1, as well as ANME-2a-d (Orphan et al. 2001; Teske et al. 2002; Meyerdierks et al. 2010; Holler et al. 2011; Biddle et al. 2012; Merkel et al. 2012; Haroon et al. 2013). The phylogenetically broadest clade is ANME-1, forming an order now named *Methanophagales* within the class *Methanomicrobia* (Adam et al. 2017). ANME-1 predominate in deeper sediment

layers that are often characterized by high sulfide and low sulfate concentrations (Elvert et al. 2005; Knittel et al. 2005; Niemann et al. 2005; Wegener et al. 2008; Roalkvam et al. 2011; Yanagawa et al. 2011; Vigneron et al. 2013). A thermophilic subgroup of ANME-1 occurs in hydrothermal sediments (Teske et al. 2002; Holler et al. 2011; Biddle et al. 2012) and hydrothermal fluids (Merkel et al. 2012). Members of ANME-1 have also been found in hypersaline seep sediments of the Gulf of Mexico (Lloyd et al. 2006). ANME-2 is a family-level group within the *Methanosarcinales*. They occur preferentially in sulfate-penetrated, sulfide- and methane-rich surface sediments (Elvert et al. 2005; Knittel et al. 2005; Wegener et al. 2008; Rossel et al. 2011; Yanagawa et al. 2011). ANME-2a are the most common and globally most widespread ANME clade (Ruff et al. 2015). They seem to have an advantage at sites where sulfate and methane concentrations are high, e.g., above shallow hydrates (Knittel et al. 2005; Lösekann et al. 2007) and in enrichment cultures (Wegener et al. 2016), and likely outcompete other mesophilic ANME lineages due to their faster growth rate. ANME-2c prefer low methane fluxes and bioturbated sediments, e.g., at seeps that are inhabited by chemosynthetic mussels and clams (Knittel et al. 2005; Felden et al. 2014). ANME-3 is a genus-level clade within the *Methanosarcinales*, and despite its global distribution, this group rarely dominates in seep sediments (Knittel et al. 2005; Lazar et al. 2011; Vigneron et al. 2013; Ruff et al. 2015). To date, the only exception is the Håkon Mosby mud volcano in the Barents Sea (Niemann et al. 2006; Lösekann et al. 2007; Ruff et al. 2019).

Sulfate reducers are commonly found in marine sediments, as sulfate is a ubiquitous electron acceptor used in the degradation of hydrocarbons or organic matter (Ravenschlag et al. 2001; Muyzer and Stams 2008). The SRB involved in mesophilic AOM belong to the class *Deltaproteobacteria* and are subdivided into several clades related to the genera *Desulfosarcina* (DSS) or *Desulfobulbus* (DBB) (Schreiber et al. 2010; Kleindienst et al. 2012) (Fig. 7). At the majority of seep sites, ANME-1 and ANME-2 are found to aggregate with the DSS clade SEEP-SRB-1 (Schreiber et al. 2010), whereas ANME-3 mainly aggregate with relatives of the DBB clade SEEP-SRB-3 (Niemann et al. 2006). These associations, however, do not seem to be exclusive, and the diversity of aggregates and mechanisms of aggregation are not well understood. Thermophilic ANME, which are a subgroup within ANME-1, form consortia with sulfate reducers affiliating with the clade HotSeep-1 (Wegener et al. 2015). The first organism of this clade “*Candidatus Desulfofervidus auxilii*” was recently cultured and described (Krukenberg et al. 2016).

Anaerobic Methanotrophy Coupled to Nitrate, Nitrite, and Metal Oxides

The anaerobic oxidation of methane coupled with sulfate reduction was a long-standing geochemical enigma until it was shown that syntrophic consortia of anaerobic methanotrophic archaea and sulfate-reducing bacteria mediate this

process (Hinrichs et al. 1999; Boetius et al. 2000). In recent years, however, evidence has emerged that methane can also be oxidized anaerobically using alternative electron acceptors. The newly described family “*Candidatus* Methanoperedenaceae” (related to ANME-2d) contains organisms that couple the anaerobic oxidation of methane to the reduction of nitrate (Haroon et al. 2013). “*Candidatus* Methylomirabilis oxyfera” belong to the bacterial candidate phylum NC10, and couple methane oxidation to the reduction of nitrite (Ettwig et al. 2010). Both processes are considered of minor importance at marine seeps since nitrate and nitrite are less abundant than the ubiquitous marine electron acceptor sulfate. However, these metabolisms appear to be widespread in freshwater ecosystems (Hu

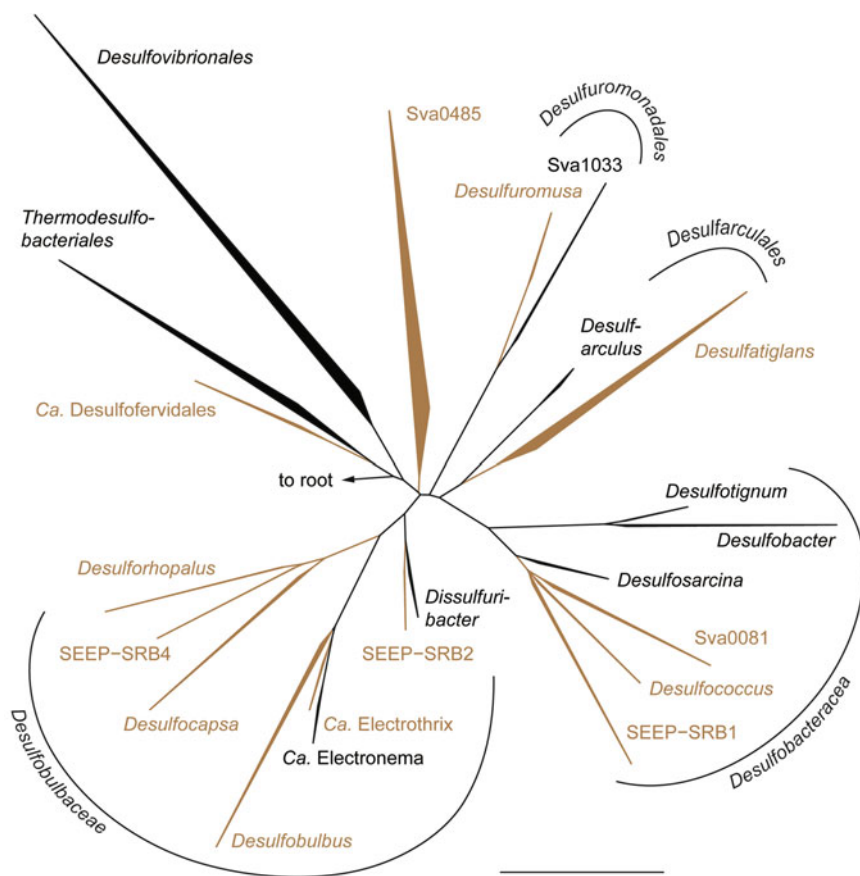


Fig. 7 16S rRNA gene based phylogeny of seep-associated *Deltaproteobacteria* (brown). *Desulfosarcina* relatives of the SEEP-SRB-1 cluster and *Desulfobulbus* relatives have been shown to associate with ANME-1, ANME-2, and ANME-3. Seep sediments also harbor *Deltaproteobacteria* that are not found in consortia with ANMEs and are common in other marine ecosystems, for example *Ca. Electrothrix* and the uncultured clade Sva0485. Scale bar: 10% estimated sequence divergence

et al. 2014; Vigneron et al. 2017; Weber et al. 2017), and in permafrost ecosystems (Winkel et al. 2018). The clade ANME-2d also includes organisms that can couple methane oxidation to the reduction of manganese- and iron-oxides (Ettwig et al. 2016; Oni and Friedrich 2017), a process that has been found to occur in coastal sediments (Egger et al. 2015) as well as in hydrocarbon seeps and hydrothermal sediments (Beal et al. 2009; Wankel et al. 2012; Sivan et al. 2014).

Aerobic and Anaerobic Oxidation of Non-methane Hydrocarbons

Although methane is generally the most abundant hydrocarbon at seeps, higher hydrocarbons can be used by free-living or symbiotic microorganisms in particular at thermogenic hydrocarbon seeps. At these seeps, the measured rates of sulfate reduction exceed those of methane oxidation (Orcutt et al. 2005), and diverse assemblages of *Archaea* and *Deltaproteobacteria* have been identified that degrade short-chain alkanes aerobically (Mastalerz et al. 2009; Redmond et al. 2010; Li et al. 2013) and anaerobically (Adams et al. 2013; Bose et al. 2013; Jaekel et al. 2013; Kleindienst et al. 2014; Dowell et al. 2016; Stagars et al. 2016). Recently, several major findings have improved our understanding of these communities. Laso-Pérez and colleagues described a consortium of the archaeon “*Candidatus Syntrophoarchaeum*” (formerly Gom-Arc87) and the partner bacterium “*Candidatus Desulfofervidus*” that couples the anaerobic degradation of short-chain alkanes with sulfate reduction, relying on biochemical mechanisms described for methanogens (Laso-Pérez et al. 2016). Rubin-Blum and colleagues showed for the first time that short-chain alkane-degrading *Cycloclasticus* are symbionts of seep-associated mussels and sponges (Rubin-Blum et al. 2017). Further details about microbial non-methane hydrocarbon oxidation are presented in Chaps. 2 and 5.

Thiotrophy

Seep-associated sulfide oxidizers—or thiotrophs—belong mostly to the gammaproteobacterial family *Beggiatoaceae* (Fig. 4 blue clades), and form extensive mats of white, yellow, or orange filaments on top of seep sediments (Fig. 3a; Joye et al. 2004; Mills et al. 2004; Knittel et al. 2005; Lloyd et al. 2010; Grünke et al. 2012; Meyer et al. 2013). The giant filamentous bacteria of the *Beggiatoaceae* are conspicuous and visible to the naked eye, as they can cover square meters of sediment. They use oxygen or nitrate to oxidize sulfide that is produced during AOM in deeper sediment layers (Preisler et al. 2007). Non-filamentous *Beggiatoaceae*, such as *Thiomargarita* and “*Candidatus Thiopilula*”, also occur at seeps, but are observed less frequently (Girith et al. 2011;