Review

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Methanogens Through Geological Time and Space: Impact on Planetary Evolution and Significance for Life Beyond Earth

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Abstract

Methanogens, also known as methanogenic archaea, are among the most ancient and widespread microorganisms, despite their particular requirements for growth. These oxygen-sensitive microorganisms have impacted climate and biogeochemical cycles throughout Earth's history, although their specific roles in the long-term carbon cycle remain little explored. Methanogens evolved early during Earth's history, likely during the Archaean Eon, in layered benthic microbial communities called microbial mats. These ancient mats, when lithified, form microbialites that represent some of the earliest evidence of life in the fossil record dating back > 3.5 Gy. Contemporary microbial mats experience a wide range of fluctuating conditions, including dramatic diel shifts in oxygen, sulfide, redox, temperature, salinity and pH. Methanogens are an integral part of marine and freshwater microbial mats and have been identified in the oxic zone of these sedimentary ecosystems; however, their adaptations to apparently unfavorable conditions and their role in long-term CO_2 sequestration through precipitation of carbonate are unclear. Furthermore, the importance and coevolution of methanogens and microbial mats may explain the global role these organisms had on Earth's major climate events during the Archean and Proterozoic eons, notably in the ending of icehouse periods and recovery of mats following mass extinctions – often in conditions with low or no oxygen. In addition to an important role in the evolution of our planet, methanogens may also produce biosignatures that are relevant for astrobiology research [and space exploration]. This review will discuss the diversity, physiology, and ecology of methanogens in order to clarify their role in biogeochemical processes through geologic time.

Keywords: methanogens; archaea; anaerobes; microbial mats; early life; exobiology; climate regulation; mass extinctions

1. The Biogeochemistry of Methanogens

1.1. Introduction

Methanogens are strict anaerobes with highly specialized biochemistry. All methanogens belong to the domain Archaea and produce methane as an end-product of their metabolism. Methanogenesis serves as their primary energy-conserving pathway, an oxygen-independent process at the limits of what is thermodynamically possible [1–4]. In addition to methane, some species also produce carbon dioxide as an end-product, contributing significantly to greenhouse gas emissions and thereby regulating the Earth's temperature [2,5], and potentially that of other planets [6].

For most of geological history, prokaryotes have dominated our planet [7] filling a diverse array of ecological niches [8,9] Methanogens are a prime, yet understudied example of an ancient and

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versatile group of microorganisms, that thrive in diverse environments, sometimes under poly-extreme conditions [3,10]. Their metabolic versatility, unique biogeochemistry, and interactions with other microorganisms, such as fermenters, syntrophs, methane oxidizers, and sulfate-reducers, may have facilitated microbial life to develop throughout Earth's history [11,12]. Furthermore, their exemplary ecological success through geologic time and space (i.e., distribution across diverse and extreme conditions) suggests that methanogens could survive conditions elsewhere in the solar system and possibly beyond, making them relevant model organisms for astrobiology [10,13].

1.2. Methanogenic Habitats

Methanogens are characterized by the production of methane (CH_4) as the obligatory product of their oxygen-independent metabolism [3]. Methanogens are found in nearly all anoxic environments on Earth, and many anaerobic niches, including deep sea, freshwater, and terrestrial sediments, marshes, swamps, permafrost soils, geothermal springs, hydrothermal vents, the rumen of cows and intestines of other mammals (including humans), arthropods, and man-made environments such as landfills, (anaerobic) digesters, and rice paddies [3,10,14–22]. Some methanogens have also been found in oxic environments as well [23], such as the surface of biofilms and microbial mats [4,23,24], the upper oxic layer of water bodies [25–27], mangroves [28], and oxygenated soils [29]. Survival of methanogens has also been assessed under a variety of exoplanetary, artificial, and space conditions, including Martian soil analogues [30,31], under simulated conditions of ocean worlds (such as Enceladus) [13], on the outside of spacecraft during interplanetary travel [10], and in clean rooms [32].

1.3. Physicochemical Boundaries of Methanogenesis

Methanogens have a unique biochemistry, which makes them well-suited for life in extreme niches [10]. As such, methanogens have been isolated from environments with a wide range of physicochemical and thermochemical conditions (**Table 1**), including: temperature [33,34], pH [3,35], salinity [16], and pressure gradients [31,34,36–38]. Some methanogens can also withstand desiccation [31], freeze/thaw cycles [33], and survive high doses of ionizing and UV radiation [39]. Methanogens are found in a wide range of extreme environments and tolerate multiple extreme conditions, making them versatile polyextremophiles [4,10,14,16]. For example, barophilic and thermophilic methanogens, members of the *Methanopyrus* genus, *Methanocaldococcus jannaschii*, and *Methanothermococcus thermolithotrophicus*, have been isolated from hydrothermal vent sites, with *Methanopyrus kandleri* strain 116 being the most heat-tolerant microorganism cultured, growing at temperatures up to 122°C and pressures up to 20 MPa [34]. On the opposite extreme, psychrophilic methanogens are abundant in wetlands and arctic permafrost [3,10] and can thrive in sub-atmospheric pressure [40]. Soda and saline lakes host alkaliphilic and halophilic methanogens belonging to the genera *Methanohalobium*, *Methanohalophilus* and *Methanosalsum* [16].

Methanogens are widely distributed and well adapted to thrive in microbial mat ecosystems [4,41–43], where they could be exposed to high and fluctuating concentrations of O_2 despite the traditional belief that they are obligate anaerobes or oxygen-sensitive microorganisms [24]. Their unique biology allows them to adapt and thrive in a range of challenging environments. For example, hydrogenotrophic methanogens, under pH and thermal stress, low H_2, iron, phosphate, and trace metals can split their metabolic investments to maintain homeostasis or add biomass, a process referred to as 'uncoupling' [13]. Some methanogens can undergo reverse reactions of some steps in methanogenesis to maximize their energy yield when substrates for respiration are sparse [15,44,45]. Such mechanisms may have played a role in their evolution through conditions in Earth past, such as through glaciations and the oxygenation of Earth's atmosphere (*see Section 2*).

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Table 1. Growth limits for methanogens and examples of species that grow at these limits. Definitions of extremes, in terms of the known limits for life [8,46] and the limits that have been identified for methanogens. The geologic relevance highlights some of the environments on early Earth, in the present, and on other planets where these conditions could be/have been found.

Environmental condition	Extremophile type (and growth definition(s))	Growth limits for Methanogens	Examples of species found within extreme	Geologic relevance
Temperature	1)Hyperthermophile (>80°C) 2)Thermophile (60-80°C) 3)Psychrophile (<15°C)	1 & 2) 122°C [2,34]. 3) -2.5°C [47].	1 & 2) *Methanopyrus kandleri* strain 116, *Methanopyrus* genus, *Methanocaldococcus jannaschii*, and *Methanothermococcus thermolithotrophicus [34].* 3) *Candidatus Methanoflorens stordalenmirensis* (freeze/thawing environments) [33], *Methanococcoides burtonii* [47].	1) Hydrothermal vents (modern day [34], potential vents of Europa and Enceladus [13], Hot springs/geothermal springs [20]. 2) Archean Oceans [48]. 3) Permafrost and ice; modern day and during glaciation events, like at the last glacial maxima; deep sediments [33,49], Terrestrial surface of Mars [36], Oceans of Europa and Enceladus [10,46].
Radiation	1) Exposure to UV 2) Ionizing radiation	1) 24 hours of 254 nm UV radiation [50].	1) *Methanococcus maripaludis* and *Methanobacterium formicicum* [50]. 2) *Methanoscarcina spp*. [50].	1) High UV radiation during the Archean [46]; surface of Mars [39]. 2) Ionizing radiation from Ur decay in nuclear geysers, possible location for the origin of life [51].
Light	1) Exposure to visible light (380-750 nm)	1) Unknown limit; growth inhibition, particularly in the blue region of the spectrum (≈370–430 nm) [52]. Reduced sensitivity in association with photosynthetic bacteria [53].	1) *Methanosarcina* and *Methanocella spp.* (observed in soil) [53]; *Methanosarcina barkeri* in a cocultured biofilm with *Synechocystis spp*. strain PCC6803 [23].	1) Surface environments on Earth (from ancient to modern day).
Salinity	1) Halophile (2-5M NaCl)	1) Moderate/extreme halophiles growth 2-3M [16].	1) Methanosarcinales *spp*., *Methanohalobium*, *Methanohalophilus* and *Methanosalsum spp*., *Methanonatronarchaeum thermophilum* and *Candidatus Methanohalarchaeum thermophilum [16,38].*	1) Archean oceans; 1.5–2 times modern salinity [54]; Saline lakes [16].
Pressure	1) Survivability to low pressure	1) Low pressure 6mbar – 143 mbar [37]. 2 & 3) 200 bar [34], 500 bar [38].	1) *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum*, *Methanococcus maripaludis* [31,36,37].	1) Terrestrial surface of Mars [37].

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	2) Barophile (obligate high pressure)/ Piezophile (adaptation to high pressure)		2) *Methanopyrus kandleri* strain 116, *Methanopyrus* genus, *Methanocaldococcus jannaschii*, and *Methanothermococcus thermolithotrophicus* [34,37].	2) Hydrothermal vents (modern day [34], and possible location for the origin of life [55]; Deep Marine Sediments [56].
pH	1) Alkaliphile 2) Acidophile	1) pH > 10.2 [3,35]. 2) pH < 3 [3].	1) *Methanohalobium*, *Methanohalophilus* and *Methanosalsum* [35].	1) Soda lakes [35].
Hydrogen concentration	1) High pH_2 2) Low pH_2 3) Fluctuations in H_2 concentration	1) > $6x10^{-2}$ bar [57]*. 2 & 3) As low as 0.1 Pa pH_2 [58]; fluctuating H_2 (from low pH_2 to overpressure) [14].	1) Species name unknown. Plausibly, early hydrogenotrophs. 2 & 3) *Methanobacterium bryantii, Methanoculleus bourgensis* MAB1, and *Methanosarcina barkeri [58]; Methanobrevibacter spp*., *Methanomicrobium spp*., and Methanosarcinales [14].	1) Earth's secondary atmosphere [57]. 2) Titan [59]. 3) Cow rumen [14]; deep biosphere [58].
Oxygen	1) Aerotolerant (tolerates some (high to low) amount of O_2)	1) Unknown limit; exposure up to 300 μM O_2 in lithifying microbial mat [60].	1) *Methanosarcina barkeri* [23]; Methanogens in microbial mats [24]. Class II methanogens [61].	1) Euxinic oceans of Proterozoic [48]; Modern day (and ancient) biofilms and microbial mats [23,24].
Metals	1) Arsenic 2) Cadmium	1) Unknown limit; Methane production by methanogens observed under high arsenic concentrations (0.8-1.5 mM) in microbial mats [62,63]. 2) Unknown limit; 10 μM of $CdCl_2$ activates the rate of methanogenesis. ≥ 100 μM of $CdCl_2$ inhibits cell growth [64].	1) *Methanomassiliicoccus spp.* (Chen et al., 2023) 2) *Methanosarcina acetivorans* [64]. Other divalent metals (Co, Zn, Cu, Fe) also activate the rate of methanogenesis at 10 μM.	1 & 2) Early oceans [65], rice paddies [66], modern day anoxic microbial mats in the Atacama, Chile [62,63].

* pH_2 of Earth's atmosphere at the time of early methanogen evolution.

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1.3.1. Temperature Extremes

Temperature on Earth through time and space shows huge variations ranging from extreme cold or psychrophilic conditions for life (growth at < 15 °C) to thermophilic (60-80 °C), and hyperthermophilic (> 80°C) conditions [8]. These temperature extremes not only exist in specific environments (e.g., modern-day hydrothermal vents [34,55]. Arctic and Antarctic permafrost and water (ice) [33,67], and deep and high-altitude sediments [49,68], but also as widespread environmental conditions of the past and present, such as during glaciations (e.g., "Snowball Earth") [69,70], through mass extinctions (rapid warming/cooling) [71], and in the Archean oceans [54]. Such extremes in temperature are also relevant for potential life in other locations in our solar system, such as on the Martian surface and in the oceans of Europa, Enceladus, and Titan [10,46] and beyond (exoplanets).

Methanogenesis, like all metabolisms, is a temperature-dependent process and therefore both substrate use and community compositions of methanogens are dependent on temperature [2]. For example, at temperatures higher than 30°C, methanogens tend to utilize H_2 to produce CH_4 rather than acetate as a growth substrate at lower temperatures [2]. Adaptations of methanogens to become psychrophiles, thermophiles, and hyperthermophiles are associated with modifications at the tRNA and protein level [10]. Psychrophilic *Methanococcoides burtonii* displays increased flexibility of polynucleotides at low temperatures associated with tRNA due to modifications with dihydrouridine [72]. The same study found tRNA modifications (e.g., methylation of nucleosides) in a thermophilic species of archaeon, *Stetteria hydrogenophila*, which can stabilize tRNA to accommodate growth under high temperatures [72]. At the protein level, *Methanococcoides burtonii* displays cold shock proteins such as Elongation Factor 2, which is unstable at higher temperatures [47]. Similarly, a so-called "Dead box" RNA helicase gene is expressed in this species at 4°C [73], a process which is also used by the thermophile, *Thermococcus kodakarensis*, when experiencing "cold" stress at 60°C [74]. Modifications are made to another cold shock protein (Csp), where the CspA homolog domain helps cells cope with cold stress [75]. Similar to adaptations in bacteria, all archaea must maintain membrane fluidity in cold environments by regulating the unsaturation of membrane lipids [76]. Low permeability and liquid crystalline phases of isoprenoid ether lipid membranes at temperatures ranging from 0-100°C are characteristic of archaeal membranes. They allow for stability at a wider range of temperatures than that found in bacteria [77]. Still, there is less membrane lipid variation observed in thermophilic methanogens than in bacteria [77].

1.3.2. UV and Ionizing Radiation

The surface of Mars experiences high doses of UV radiation from the sun, which may restrict extant life to the subsurface and could erase surface biosignatures of ancient life over geologic time [78]. Life on the Archean Earth also experienced higher levels of UV radiation due to the lack of an ozone layer in the upper atmosphere [79]. This condition favors life to develop and evolve in microbial mats [4]. Exposure to UV radiation leads to the limited survival of *Methanococcus maripaludis* and *Methanobacterium formicicum* under simulated Mars conditions [39]. While the exact mechanisms by which methanogens adapt to UV stress are unknown, DNA repair mechanisms could play a role [39]. Physical barriers can protect cells from UV radiation, including FeS, which was abundant on early Earth [48], in layers of soil and sediment surrounding the cells [39], through exoplymeric substances (EPS), and in photopigments in microbial mats containing methanogens [4,69]. *Methanoscarcina spp*. survives radiation and desiccation [50] by deploying enzymes and DNA repair mechanisms to protect against oxidative stress [50].

1.3.3. Visible Light

The sun's luminosity has increased through geological time, while short-wavelength radiation has decreased [80]. Light that reaches the Earth's surface is a critical environmental factor that

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regulates a wide range of biological processes. At the molecular level, visible light (wavelengths: from 380 to 750 nm) is perceived by sensory photoreceptor proteins that detect specific wavelengths and convert light signals into biochemical responses [81,82]. These photoreceptors span a broad region of the electromagnetic spectrum, ranging from near-ultraviolet (near-UV) to near-infrared (NIR) wavelengths [82].

In methanogens, visible light from both solar and artificial sources negatively affects growth, morphology, and physiology, and in some species, it can even trigger cell lysis and death [52]. Conversely, the absence of visible light has long been considered a critical parameter for the optimal cultivation of multiple strains of methanogens isolated from diverse environments [83–85].

Recent mesocosm experiments, conducted in artificial ponds containing environmental sediments, showed that methane emissions by methanogens are two- to threefold higher at night compared to daytime levels [86–88]. A decade-long monitoring of microbial diversity and functional activity demonstrated that sunlight modulates the production rates of methane and carbon dioxide, indicating sunlight-dependent metabolic changes in methanogens [88]; however, it is still unclear whether this effect is due to temperature fluctuations or direct exposure to sunlight.

Despite their sensitivity to visible light, methanogens coexist with oxygenic photosynthetic bacteria in various natural habitats, including microbial mats, soil crusts, and the aerobic epilimnion of oligotrophic lakes [23,24,53,89–91]. The coexistence of methanogens and phototrophs may be more common and widespread in nature than previously recognized [23,92,93]. It is likely that anoxygenic photosynthetic bacteria act as photosensitizers, promoting the CO_2-to-CH_4 conversion in association with anaerobic methanogens when cocultured in anoxic biofilms [23]. Taken together, these observations suggest that visible light exerts a far more complex influence on methanogens than previously understood, not only constraining their physiology but also enabling novel ecological interactions.

1.3.4. Salinity

Methanogens in the Archean would have evolved in an ocean 1.5-2 times more saline [54] than the average of 35g.kg^{-1} today [94]. Higher salinity would have affected not only the ecophysiology and evolution of methanogens but also could have aided in the warming of the early oceans [94].

There are two primary mechanisms archaea utilize to maintain osmotic balance in halophilic environments. The first is a so-called 'salt out' approach in which an organic osmotic solute, such a glycine betaine, dimethylsulfoniopropionate, ecotine, or hydroxyctine is utilized internally by the cell to maintain lower cellular concentrations of NaCl than in its surroundings [16,43,95,96]. These compatible solutes provide osmotic protection for the cell through the removal of salts without interfering with biochemical processes [16,97]. The second is a 'salt in' method, through high intracellular concentrations of K^+, which is employed by species of the genus *Methanonatronarchaeum* [38,95]. This genus contains extremely halophilic methanogens species *Methanonatronarchaeum thermophilum* and *Candidatus Methanohalarchaeum thermophilum,* which are novel in terms of their metabolism, utilizing C_1 compounds as an electron acceptor and H_2 as an electron donor for methanogenesis [16]. These species have abundant UspA family stress response proteins, their membrane lipids contain pleckstrin homology domains, and archaeal histones may reinforce the stability of RNA, DNA, and proteins – all of which may aid in their adaptations to high salt concentrations [16].

1.3.5. Pressure

Studies simulating low-pressure conditions on Mars reveal the survival of methanogens in 6-400 mbar [36,37]. The physiological and physiochemical adaptations of methanogens in low-pressure environments are unknown, due to the lack of relevance in Earth environments. Survival in low-pressure environments is in part attributed to the rigidity and structure of archaeal lipid membranes [36]. Some species, like *Methanoscarcina* are flexible in their electron donor choice and grow in

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multicellular clusters, which makes them more resistant to desiccation and possibly low gas pressures [36].

High pressure, or barophilic conditions, are better studied in the context of the Earth's biosphere, for example, in the deep-sea or in oceanic and terrestrial subsurface sediments [56]. Barophilic pressure conditions often coincide with other extremes, such as hyperthermophilic conditions at hydrothermal vents [34,38,56]. Morphological adaptations to high temperatures are thus present in methanogens growing at high pressures [38,56]. Some species, like *Methanococcus thermolithotrophicus*, typically coccoidal cells, have been observed to grow in an elongated shape under high hydrostatic pressure [38].

1.3.6. Hydrogen Concentration

Low and high partial pressure of hydrogen (pH_2) is found in a variety of environments on Earth and is highly relevant in astrobiology, such as the low pH_2 atmosphere of Saturn's moon Titan [59]. A high pH_2 was characteristic of Earth's early secondary atmosphere, with a pH_2 of over $6x10^{-2}$ bar [57]; conditions under which early methanogens evolved. The evolution of nitrogen fixation over 3.2 billion years ago [98] may have contributed to an abundance of H_2 [99], which could have supported early methanogenesis.

At the community level, methanogens adapt well to fluctuations in H_2 found in the rumens of cows and sheep, which change from low concentration H_2 to supersaturated conditions [14,100]. Some of the species found in these communities, like *Methanosarcinales*, lack cytochromes and are associated with short doubling times and mesophilic temperatures [14]. Lithotrophic methanogenesis (i.e., use of H_2/CO_2; *see Section 1.4.1.3*) is most common in gut communities [14]. Trace metals, such as nickel used in cytochromes, may aid in adaptation to low H_2 conditions [58].

1.3.7. Oxygen Exposure

Methanogens are considered oxygen-sensitive microorganisms and obligate anaerobes, but are exposed to high levels of oxygen in modern-day microbial mats [4,24] and potentially in oxic environments such as surface waters, including those of the Proterozoic eon. Presence of O_2 in the environment expanded the redox states of elements and allowed for greater variation in electron donor/acceptor availability (i.e., aerobic metabolism) [101]. Exposure to O_2 in biofilms [23], microbial mats [24], and other terrestrial and aquatic systems [25–27,29] may challenge the doctrine that methanogens are strict obligate anaerobes: some species are clearly more aerotolerant than previously thought [102]. Ye and colleagues (2024) observed enhanced methanogenesis in lab-grown cocultured biofilms of a cyanobacterium, *Synechocystis* PCC6803, and a methanogenic archaeaon, *Methanosarcina barkeri.* During dark cycles, syntropic methanogenesis was driven by inorganic products (H_2/CO_2) produced through respiration by cyanobacteria, while *M. barkeri* reduced the pH_2, enhancing *Synechocystis* PCC6803 growth [23]. During the day, active photosynthesis oxygenated the biofilm, triggering the generation of reactive oxygen species (ROS) by *M. barkeri*, which oxidized methyl radicals, eventually resulting in the abiotic production of CH_4 [23,103]. The ROS produced in the presence of O_2 can also be used as an organic substrate for CH_4 production by *M. barkeri* [23]. Additionally, diel fluctuations in metabolic activity by *M. barkeri* established Fe-redox cycling, which enhanced growth [23].

1.3.8. Metals

The abundance and redox state of elements have changed radically through Earth's evolution, predominantly due to the oxygenation of the oceans and atmosphere [65]. Methanogens can transform the redox state of metals in processes that lead to their solubilization or biomineralization [104]. This dynamic interaction is a response to their need for certain elements to carry out their physiological functions [105]. A considerable number of metals are indispensable for cellular function, given their well-established biological roles. These include Fe, Na, K, Ca, Cu, Zn, Mn, Mo,

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Co, and Mg [106]. Collectively, these elements constitute the basis of cellular metabolism and are fundamental to processes such as enzymatic catalysis, biological polymer folding, osmoregulation, membrane transport, and signal transduction [107]. Consequently, intracellular biochemistry can produce a "chemical fossil record" that reflects the geochemical conditions of the early Earth [108]. The use of metals can be viewed as a primitive metabolic strategy that has influenced the evolution of protocells since the very origin of life [109]. Indeed, all classes of biological molecules, from nucleic acids to proteins and antibiotics, interact with metal ions, which explains why living cells invest considerable effort in tightly regulating their intracellular concentrations [107].

There is strong scientific evidence that metals (both essential and non-essential) play a crucial role in the global carbon cycle, particularly in the production of methane and the consumption of CO_2 [64,110–115]. All methanogens require Fe, Ca, Co and Ni for optimal growth, while some also need Zn, Se, Mo and/or W [111,116,117]. Iron can serve as a carrier or reactant in electron transfer, especially in the form of iron-sulfur ([Fe-S]) clusters [105]. The greater reliance on [Fe-S] clusters in methanogens has been suggested to result from increased bioavailability of Fe and S in anoxic early Earth environments (*see Section 2.1)* [118]. Nickel is an important cofactor present in methyl coenzyme M reductase (Mcr); the final enzyme in the methanogenesis pathway, and is a key component of the cofactor F_{430} [111]. One hypothesis suggests that the decline in nickel availability around 2.7 billion years ago contributed to the reduction of atmospheric methane levels, thereby weakening the methane greenhouse and paving the way for the Great Oxidation Event (*see Section 2.2)* [119,120]. Cobalt is found in corrinoid cofactors (cobalamides), which are present within methyltransferases required for the transfer of the methyl group from methyl-tetrahydromethanopterin (methyl-H_4MPT) to coenzyme M (CoM) [121]. Calcium and magnesium are required at low concentrations for methanogenic growth [122,123], but at high concentrations disrupt cellular processes, such as quorum sensing (i.e., cell-to-cell communication) [124] and electron transport [125]. Zinc is present in the heterodisulfide reductase (Hdr), where it plays a structural and catalytic role in the reduction of CoM-S-S-CoB, thereby recycling the cofactors required for the activity of the methyl-coenzyme M reductase enzyme [126]. Molybdenum is involved in catalyzing the first step of hydrogenotrophic methanogenesis through formylmethanofuran dehydrogenase, as well as the Wood-Ljungdahl pathway of CO_2 fixation. Similarly, the oxidation of formate to CO_2 and hydrogen (H_2) is catalyzed by formate dehydrogenase, which is Mo-dependent [127,128]. Under conditions of Mo limitation, tungsten can substitute for molybdenum due to its similar chemical properties [129].

Methanogens exhibit distinct responses to heavy metals. Although some of these are required for growth, others are well-known for their inhibitory effects on these microorganisms. Heavy metals are generally defined as metallic elements with a high atomic weight and a density at least five times greater than that of water [130]. The term "heavy metals" refers to both essential and nonessential trace elements, which may exert toxic effects depending on their intrinsic properties, chemical speciation, and concentration [131]. This definition classifies cadmium, copper, chromium, mercury, nickel, lead, and zinc as heavy metals. The abundance of these heavy elements in the dissolved state may have supported the evolution of methanogens early in Earth's history [65]. It should be noted that the term "heavy metals" includes certain metalloids, such as arsenic and selenium, based on the historical assumption that heaviness correlates with toxicity [130]. As a consequence of such long-standing interactions, microorganisms are equipped at least with one As-detoxifying mechanism to prevent its toxicity [132]. This phenomenon may reflect the central role of arsenic toxicity in the evolution and survival of life throughout Earth's history [133–135]. Methylation of arsenic, a common detoxification pathway, has been documented in methanogens [66]. Although arsenic can inhibit aceticlastic and hydrogenotrophic methanogenesis [136,137], recent reports indicate that arsenic can also promote aceticlastic methane production at low concentrations (10μM) [138]. The effect of As on methane production seems site-specific. For example, in permanently anoxic microbial mats in the Atacama, Chile, methane was produced in the presence of 0.8-1.5 mM total arsenic [62,63]. A similar stimulating effect has been observed with Cd, which can increase methanogenesis rates up to nine-fold in pure cultures at concentrations of 10 μM [139,140]. In contrast, metals such as copper (Cu) and

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chromium (Cr) inhibit methanogenesis, although they do not necessarily lead to a significant reduction in biomass [111,141].

1.4. Evolution and Diversity of Methanogenic Pathways

As mentioned, methanogenesis is one of the oldest metabolisms on our planet, with estimates placing the onset no later than 3.8 to 3.5 Gya [6,48,142,143] in Earth's secondary, pH_2-rich atmosphere [48,94]. Phylogenetic analyses suggest that methanogenesis may have evolved earlier, possibly around 4.1 to 3.8 Gya [7], during the late heavy bombardment [144]. They diverged within the kingdom Euryarchaeota ~3.51 Gya [143] based on estimates of the date of horizontal gene transfer (HGT) events between methanogens and cyanobacteria, which were calibrated to chemo- and microfossil evidence [143]. There is limited geochemical evidence of methanogenesis before their divergence within Euryarcheota in the Archean. To date, the only geochemical evidence that places the evolution of methanogenesis during the Archean is CH_4-rich fluid inclusions in metamorphosed rocks from the Pilbara craton, dated back to ~3.5 Gy [142] and confirmed by Missbach and colleagues [145], as well as from isotopic signatures of ^{13}C from ~2.7 Ga-old kerogens [146]. Lipid biomarkers of archaeal membranes have been identified in the rock record dating back to 2.7 Gy [147]. However, certain abiotic processes could produce similar isotopic signatures, and the biogenicity of methane in Archean samples remains unresolved [62].

1.4.1. Methanogenic Pathways

Methanogens are a diverse group of microorganisms that belong to several different taxa within domain Archaea [10,143,148]. Methanogens produce CH_4 anaerobically [3], however, they are not the only microorganisms to produce this gas [103].

There are three primary pathways associated with methanogenesis, also called the Wolfe-cycle (*see Section 1.4.1*), but methanogens are often metabolically flexible (i.e., mixotrophic), with some capable of utilizing more than one energy-generating pathway. For example, *Methanoscarcina acetivorans* is a model organism for aceticlastic methanogenesis, but can also grow on certain C_1 compounds, such as methanol, methylamines, and methyl sulfides [15]. While methanogenesis lies on the thermodynamic threshold of life ($\Delta G^{0'} = -20$ kJ mol^{-1}) [3,149,150], the energetic yield can be modified by combining methane production with other metabolic reactions. Coupling of one of the primary methanogenic pathways with other microbial metabolisms increases the overall energy yield, such as in syntrophic methanogenesis (e.g., with cable bacteria) ([11,151,152], interspecies hydrogen transfer [153,154], and reverse methanogenesis coupled to sulfate reduction (anaerobic methane oxidation) [154,155]. Energetic yield of the various reactions varies, depending on temperature and actual concentration of the reactants and products [2,150,155].

1.4.1.1. Aceticlastic Methanogenesis

Aceticlastic (also referred to as acetoclastic) methanogenesis is perhaps the most common form of methane production in the environment [156,157], as acetate is likely abundant in many habitats [2,15,156]. The aceticlastic pathway is a chemoorganoheterotrophic process of methane production that utilizes acetate ($\Delta G^{0'} = -11.0$ kJ mol^{-1}) [150] or acetate-like compounds, such as pyruvate or propionate (Equation (1)) [3,150,158,159]. For detailed information on the thermodynamics for various growth substates, *see* [15,150].

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (1)$$

1.4.1.2. Methylotrophic Methanogenesis

C_1 compounds (e.g., methanol, methylamines, methyl sulfides and thiols, methyl halogens, and methylated aromatics) get reduced through the methylotrophic pathway (Equation (2)) (another mode of chemoorganoheterotrophic growth) [3,150,158,159]. The energy yield of these compounds

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depends largely on the number of methyl groups available; for example, the $\Delta G^{0'}$ of methanol is -290.0 kJ mol^{-1}, dimethylamine is -167.0 kJ mol^{-1}, and trimethylamine is -510.9 kJ mol^{-1} [2,150].

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \quad (2)$$

1.4.1.2. Lithotrophic Methanogenesis

Lithotrophic methanogenesis (i.e., hydrogenotrophic methanogenesis) is a chemolithoautotrophic pathway where CO_2 is reduced (from CO_2(g) or HCO_3^-/CO_2 (aq), $CaCO_3$ (S), carbon monoxide, formate, or some alcohols paired with CO_2) using H_2 as an electron source (Equation (3)) [150,160–162]. In some cases, calcium carbonate is used as a source of electron acceptor (i.e., as source of CO_2) [160]. The $\Delta G^{0'}$ of equation 3, using H_2(g) as an electron donor, is -193.0 kJ mol^{-1} [2,150].

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (3)$$

In terms of their evolution, hydrogenotrophic pathway are likely more ancient than aceticlastic and methylotrophic methanogenesis [48]. While methylotrophic methanogenesis was likely next to evolve [48]. Hydrogenotrophic methanogenesis may have been supported by the abundance of H_2 in an early anoxic atmosphere. Estimates for Archean atmospheric hydrogen concentrations range from a conservative 1000 ppm [48] to 10,000 ppm [5] or even 300,000 ppm [163], which are all well above modern levels of 530 ppb [164]. Lithotrophic methanogens also share hydrogenases with the Last Universal Common Ancestor (LUCA), which suggests LUCA likely utilized H_2 as part of its metabolism [48,165,166].

1. Signatures of Methanogens Through Geologic Time

The ecophysiological background of methanogens reviewed above is instrumental in understanding the impact that these microbes have had on the evolution of our planet. From early Earth to modern time, methanogens have altered Earth's biosphere, atmosphere, and lithosphere. While there are challenges associated with biogeochemical evidence of methanogenesis in deep time (i.e., through the Archean and Proterozoic eons), indicators of methanogen activity in the rock record include depleted $\delta^{13}C$ isotopes -27‰ to -38‰ [167], of which the activity of biological methane oxidation (both anaerobic and aerobic) can alter these signatures [48], further fractionating ^{13}C isotopes [146]. In some cases, the isotopic fractionation effect deviates, e.g., hyperthermophilic methanogenesis in environmental extremes of high heat (~122°C) and hydrostatic pressure (> 40 MPa), yields a fractionation of < −12‰ [34]. Methanogen-specific lipids have been used as an indicator of methanogens in sedimentary structures, both ancient and modern [142,168,169]. It should be noted that abiotic fractionation can also produce a negative $\delta^{13}C$-methane value. In Fischer-Tropsch synthesis reactions, the methane that forms can have isotopic C values as low as -45‰, depending on the iron mineral used in the reaction [170].

2.1. The Archean Eon (4-2.5 Gy)

The atmosphere of the Archean was defined by pervasive anoxia. Methane was delivered to early Earth's atmosphere by volcanism (which released H_2, CH_4, CO_2, and NH_3), metamorphic reactions such as serpentinization (Equation (4)) [171] and Fischer-Tropsch synthesis reactions, which often concur in hydrothermal vents. (Equation (5)) [170], and through impacts with comets and asteroids [6,172], but in addition, and largely, through biogenic CH_4 production [6,48,142,167].

$$6Fe_2SiO_4 + 7H_2O \rightarrow 3Fe_3Si_2O_5(OH)_4 + Fe_3O_4 + H_2 \quad (4)$$

$$(2n+1)H_2 + nCO \rightarrow C_nH_{2n+2} + nH_2O \quad (5)$$

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Large amounts of microbially-produced methane in the atmosphere (100-15,000 times present concentrations) (**Figure 1A**) [48] made up a major component of the carbon cycle and contributed to a greenhouse effect along with high atmospheric pCO_2 (0.6 bar) [57]. This obviously would have supported early methanogenesis [173]. The high concentration of greenhouse gases kept global temperatures elevated [48,174], despite 20-25% lower solar radiation than today [48]. Secondary ion mass spectrometric analysis of kerogens recovered from 3.4 Gy Apex chert of Northwestern Western Australia revealed isotopic signatures consistent with methanogenesis, suggesting that this metabolism was likely an important process in the Archean biosphere [167]. This is consistent with the earliest evidence of methanogenesis from hydrothermal deposits dating back to 3.5 Gy [142].

2.1.1. Sulfur Cycling

The Archaean oceans contained high levels of iron sulfides (FeS and FeS_2), hydrogen sulfide (H_2S), zero-valent sulfur (S^o), and possibly thiosulfate ($S_2O_3^{2-}$) [48,175]. All these reduced sulfur compounds can fulfill the nutritional requirements for methanogens [48,176,177]. Sulfur fractionation occurs during enzymatic S^o-reduction with H_2 [178]. Low sulfate and thus the absence of sulfate-reducing microbes in the early Archean [179,180] as well as a lesser degree of $\delta^{34}S$ fractionation observed in the fossil record from that Eon [178], has been used to corroborate an early (i.e., Paleoarchean) presence of assimilatory sulfur reduction by methanogens [48]. Furthermore, proteins of lithotrophic methanogenic species that contain Fe-S clusters are thought to be among the earliest inorganic catalysts, as they can self-aggregate in high concentrations, like those found in the iron and sulfide-rich oceans [48].

In addition to inorganic sulfur, organic sulfur compounds made up an important part of the cycling of this element during the Archean as well [181]. Both biotic and abiotic reactions produce low-molecular-weight organic sulfur compounds [43]. Of these organosulfur compounds, methanethiol (CH_3SH) and dimethylsulfide ($(CH_3)_2S$) are important sources of electrons during methane generation in microbial mats [42,43]. Vast amounts of methylated sulfides were produced by volcanoes and hydrothermal vents during the Hadean and Archean [182], and could thus have supported the evolution of methylotrophic methanogens early during Earth's history. A reconstruction of microbial metabolisms in microbialites of the Dresser Formation, Pilbara, Australia, suggests that sulfate- and arsenate-reducing microbes may have competed with methanogens for metabolic substrates [183], a notion is based on the presence of sulfate and arsenic in these 3.5 Gy-old microbial sediments. Demethylation of arsenic is not only a detoxification mechanism, but in some cases, it can also support methane production, thus generating energy in methylotrophic methanogens [184].

2.1.2. Environmental Conditions and Photochemistry

Methanogens in the Archean would have experienced several 'extreme' conditions, like increased temperatures [54], higher amounts of UV surface radiation [79], and higher salinity in the oceans [54]. This may have been 'normal' for methanogens that had evolved at the time. Some localized environments may have hosted methanogens and could have been potential locations for the origin of life, such as hyperthermophilic conditions at hydrothermal vents [55] or exposure to ionizing radiation in nuclear geysers [51]. The average global ocean temperatures were in the range of 55 to 85°C and could have supported thermophiles as potentially the most ancient extremophilic mode of life [54]. This is further supported by some studies that suggest that LUCA could have been a thermophilic microbe [166,185]. Alternatively, studies focusing on hydrogen and inorganic phosphate ($\delta^{18}O_P$) isotopes report a much lower ocean temperature of 26 to 40°C [186,187]. Under this scenario, or if it evolved in a non-marine system, LUCA may have been a mesophile. A mesophilic

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origin of LUCA is also supported by the thermoreduction hypothesis [188]. Increased ocean temperatures could have a greater potential for methane production from hydrogenotrophic and mixotrophic methanogens [189]; however, an ocean temperature of 40°C could have potentially been (near) optimal for methane production from both aceticlastic and hydrogenotrophic species [2,189].

Exposure to UV radiation at the surface of the planet was greater during the Archean due to the lack of ozone in the upper atmosphere [79]. Physical barriers to this radiation, perhaps protected microbes in the ocean, such as the high abundance of iron [39]. A variety of photopigments in microbial mats and microbialites could have quenched this damaging high-energy radiation [4]. Salinity reconstructions of the Archean oceans suggest that salinity was 1.5 to 2 times higher than it is at present [54], which would have supported halophilic species.

The impact methanogens had on the Archean carbon cycle not only affected the increased global temperature at the time but had a lasting effect on the evolution of Earths secondary and tertiary atmosphere as methane likely supported the irreversible oxidation of the atmosphere, oceans, and terrestrial systems in the Proterozoic [48]. Methane was removed in the Archean atmosphere by photochemical breakdown of CH_4 into 4H and C by UV radiation, providing a source of OH radicals in the upper atmosphere [48,190] (Equation (6)).

$$CH_4 + uv \rightarrow C + 4H \quad (6)$$

While it is still debated, anaerobic methane oxidation (possibly by methanogens and other heterotrophic acetogens [15,191]) coupled to sulfate-, arsenate-, nitrite- or iron reduction [183,192] could have played a role in the removal of methane in the late Archean [146] (**Figure 1B**). Methanogens may also have been critical in the formation of an organic haze. When the atmospheric ratio of CH_4:CO_2 exceeds 0.1, a photochemical reaction of these gases using energy from UV radiation produces a variety of n-alkanes [193]. Atmospheric photolysis of CH_4 and N_2 formed HCN and $NH_4(OH)$ [194], adding an essential nutrient for life to the global oceans. This Archean organic atmospheric haze was not as thick as, e.g., the current haze on Saturn's moon Titan (which has a CH_4:CO_2 ratio > 1) and may have had a climate-stabilizing effect on our planet [195].

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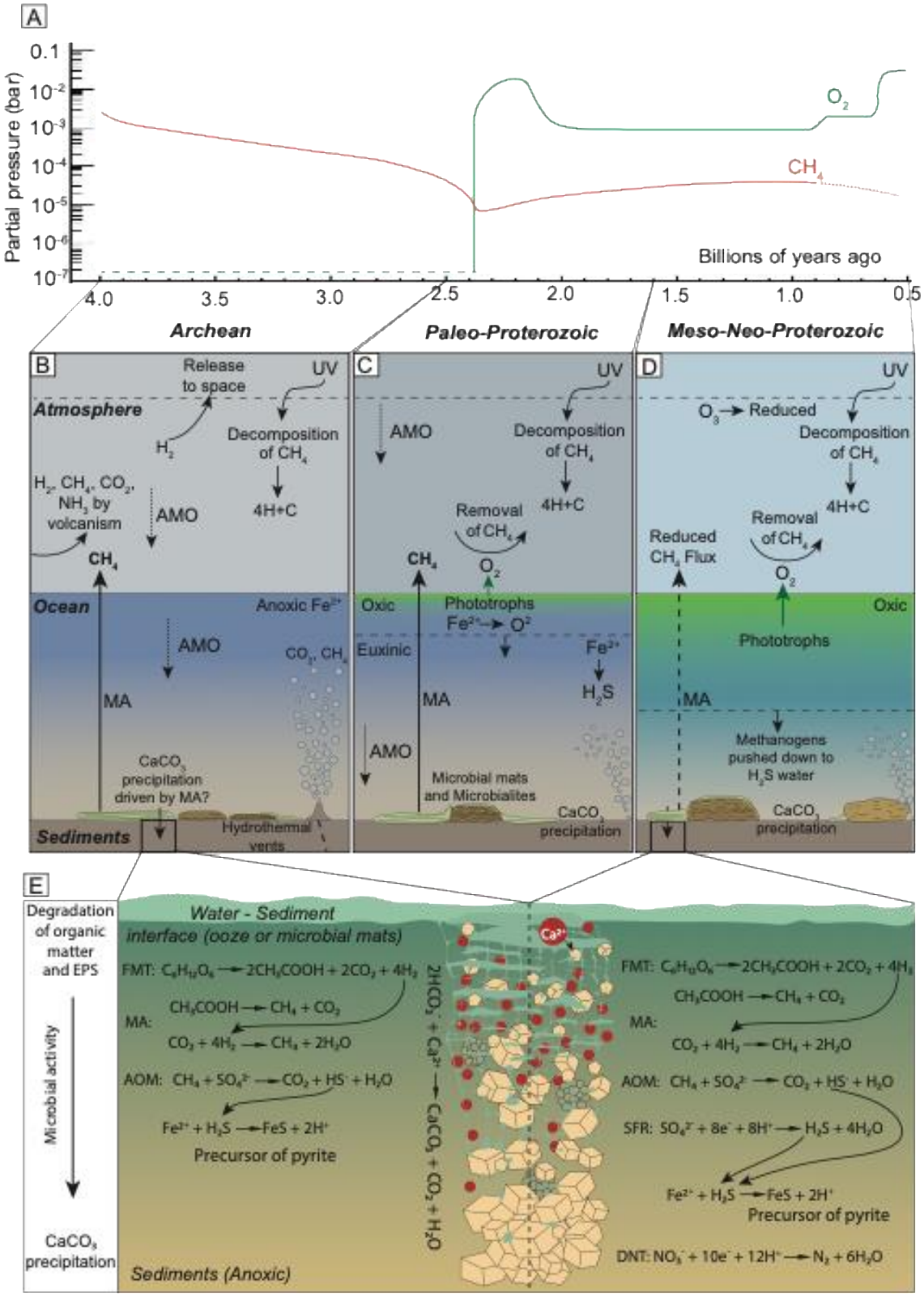

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Figure 1. Methane in the carbon cycle during the Archean and Proterozoic Eons. **(A)** Atmospheric CH_4 and O_2 concentrations from ~4.0 Gy to 0.5 Gy, adapted from [57] **(B)** The role of CH_4 in the iron-rich oceans of the Archean, when there was a large flux of CH_4 from methanogenesis (MA) in the oceans; sinks of methane included photochemical breakdown in the upper atmosphere and possible anaerobic oxidation of methane (AOM). The CH_4 production exceeded removal, which resulted in a strong greenhouse effect and warming characteristic of the Archean atmosphere. Arrows show the cycling of CH_4, including the flux of CH_4 to the atmosphere by MA and the removal and decomposition of CH_4. **(C)** CH_4 and the carbon cycle in the Paleo-Proterozoic. Oxygen produced by cyanobacterial photosynthesis emerges as a novel oxidant for microbial removal (sink) of CH_4 from the atmosphere. **(D)** Atmospheric CH_4 is further reduced during the Meso-Neo-Proterozoic due to increased removal by oxic and anoxic methane oxidation, i.e., reverse methanogenesis coupled to sulfate and/or arsenate reduction, as well as through photochemical reaction of CH_4 with O_3 (through OH^-). **(E)** CH_4 production by syntrophic microbial processes through carbon degradation in sediments during the Archean and Meso-Neo-Proterozoic Eons. H_2, and organic products (such as CH_3COOH), from fermentation (FMT) could have supported MA in sediments. Anaerobic oxidation of methane (AOM) by sulfate reduction (SRF; shown here, but possibly also respiration using other electron donors such as nitrate, selenate, or arsenate (*see Section 1.3.8*) could have contributed to the removal of CH_4 in sediments and promoted mineral formation (such as (arseno)pyrite). Active MA combined with other microbial processes [196] can drive carbonate mineral precipitation (such as $CaCO_3$) in sediments and microbial mats.

2.1.3. Methanogens in Early Hydrothermal Vents

Hydrothermal vents were important sites for the evolution and diversification of early life [55,183,197–199]. The oldest fossilized hydrothermal vents date back 440 My [200], but these sites must have existed much longer, possibly 4.3 Gya [201]. Vents (fluid) of the Archean were likely highly alkaline (pH 8.5 to >12) [202] and high temperature (~290 to 350°C) [183,198], vastly different than the black smokers (350°C to >450°C; pH 2-3) and white carbonate chimneys (50-90°C; pH 9-11) found in today's oceans [55]. Similarly, the physicochemical gradient of vent fluid, in their interaction with the surrounding neutral to acidic seawater, was the reverse of what is observed today, where vent fluids interact with a more alkaline ocean [198]. Still, modern-day vent systems may provide relics of ancient biogeochemical interactions (for further reading, see [55]).

Archean vent fluids were rich in iron and sulfide [183,203], CO_2 and $CaCO_3$ [198], and SiO_2 [198]. While the Fe content of ancient vent fluid and, likewise, a hydrothermal contribution to Archean banded iron formation is debated [198,204], serpentinization in vent fluids supplied abundant H_2, C_{1-4} intermediates, and metals which could have supported hydrogenotrophic methanogens, acetogens, and sulfate reducers as early metabolisms [166,199,205,206]. For example, transition metals Co, Ni, and Fe from vents could have been a source of early cofactors in the acetyl-CoA (Wood-Ljungdahl) pathway found in modern-day methanogens, acetogens [205,207], and possibly in LUCA [166,199]. Additionally, some methanogens can fix N_2 using dinitrogenase reductase [208]. Such nitrogenases are ancient and were likely important for early biochemistry at hydrothermal vents [199]. Mo may have also been abundant in Peridotite-hosted alkaline systems [209]. Hydrothermal activity in the Archean could have also been a source of oxidized compounds, such as SO_4^{2-}, which could have expanded element cycles and the potential for microbial metabolisms (i.e, sulfate reduction) [183,202].

2.1.4. Microbial Mats of the Archean

Microbial mats are semi-closed sedimentary biofilms comprising bacteria and archaea, in a matrix made up of extracellular polymeric substances [4]. Microbial mats in the Archean were likely dominated by H_2S –including methanogens and perhaps methane oxidizers, as well as other early heterotrophs [4,63,183,210–212]. However, it is thought that the earliest microbial mats may have been chemolithotrophic rather than autotrophic [4,63,183]. Microbial mats could have been among the earliest stable habitats [212], and the combination of a complex geochemical composition, a high

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cell density, and a close proximity of organisms would have made them ideal sites for the evolution of early life [4,63,212] and the diversification of metabolisms [183]. At the onset of the Neoarchean, cyanobacteria in microbial mats combined two photosystems [213] and the oxygenation of Earth's oceans, atmosphere, and protocontinents took off [214]. Although the cyanobacteria date back ~ 2.8 Gy, their photosynthesis can also be supported by reduced sulfur species, and thus their first appearance can not be used as evidence for oxygen production. In fact, anoxygenic photosynthesis (e.g., using reduced sulfur as an electron donor) likely prevailed for tens of millions of years in these phototrophs [215]. Microbially-induced sedimentary systems [216] and stromatolites [217] record the preservation of microbial mats in the fossil record. The earliest evidence for this is found in the Pilbara dating back ~ 3.5 Gy [210,218,219]. Even though the true nature of the 3.7 Gy-old stromatolitic structures remains to date under discussion [220–222], these fossilized lithified microbial mats, which include stromatolites, thrombolites, dendrolites, and leiolites, are the main systems that provide insight into the biogeochemistry of the Archean world [4,217,223].

2.2. The Proterozoic

2.2.1. The Great Oxygenation Event and the Paleoproterozoic (2.5 to 1.6 Gy)

The evolution of oxygenic photosynthesis ~2.5 Gya spurred the permanent oxidation of the planet (**Figure 1A**) and led to greater oxidation states of the sulfur cycle [48,175] and expansion to the carbon cycle through the evolution of new metabolic pathways [48]. The abundance of methane left over from the Archean likely contributed to the irreversible, but slow, oxidation of the atmosphere, terrestrial environments, and Earth's oceans [179]. Anoxia in the atmosphere was prolonged due to icehouse conditions [224]. Reduced atmospheric CO_2 could have contributed to quicker removal (through oxidation) of methane by O_2 [224] (**Figure 1C**). This relatively rapid removal of CH_4 likely contributed greatly to/was an important factor in the reduction in global temperatures [48,174]. The oxidation of sulfide increased the sulfate concentration in the oceans and set the stage for the evolution of sulfur metabolisms 2.3-2.4 Gya [175].

2.2.2. The Boring Billion (1.8 to 0.8 Gy)

The onset of oxidation of reducing pre-GOE conditions resulted in euxinic, stratified oceans with oxygenated surface waters and sulfidic, anoxic bottom waters [48]. Anoxic oceans contained an abundance of hydrogen sulfide, which some methanogens used as a sulfur source [48]. Reactions between the oceanic ferrous iron (Fe(II)) and atmospheric O_2 lead to the precipitation of iron sulfide minerals, like pyrite. This limited the availability of Fe(II) and other trace metals, such as nickel [225], which are essential for methanogen growth [58]. Some species of methanogens can reductively dissolve nickel and iron sulfide minerals, making them more bioavailable in euxinic conditions [112]. This capacity could have been used to survive in the anoxic zone of the oceans during the Boring Billion, which would have supported a continuing methane flux to the atmosphere [174]. However, methane concentrations were presumably reduced to less than 10 ppm in the mid Proterozoic [174] from Archean levels in the magnitude of 10^2 to 10^3 ppm [48,190], either through removal of large amounts of CH_4 from the atmospheric reservoir and/or a reduction in CH_4 flux to the atmosphere [174].

Sulfate reduction-supported anaerobic oxidation of methane could have limited the impact of methanogenesis on the global climate [174], in which CH_4 is removed via anaerobic methane oxidation (**Figure 1E**) (Equation (7)):

$$CH_4 + 2H_2O + OH^- \rightarrow HCO_3^- + 4H_2 \quad (7)$$

Sulfate-reducing bacteria (SRB) consume H_2 (Equation (8)) produced during methanogenesis, lowering the availability of this for methanogens [45]:

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$$4H_2 + SO_4^{2-} \rightarrow HS^- + OH^- + 3H_2O \quad (8)$$

A similar role in anaerobic methane oxidation has been attributed to arsenate, iron, manganese, and nitrate reducers (*see Section 1.3.8*). Anaerobic methane oxidation thus created a sink for methane in the Proterozoic oceans (**Figure 1D**), removing most of this gas before it could escape into the atmosphere [174]. Simultaneously, methane removal by UV (Equation (6)) slowed down when oxygenation (and consequently O_3 production) of the atmosphere commenced. These two events combined may help explain the steady but severely reduced temperature during the Boring Billion [226].

2.2.3. Methanogens Through Glaciations

2.2.3.1. The Ending of Marinoan "Snowball" Earth (650 – 635 Mya)

The continual decrease in the greenhouse gas concentration, including CO_2 and CH_4, through the mid-Proterozoic and Neoproterozoic eras triggered extensive low latitude glaciation, and perhaps the most extensive ice age of the planet [69,70]. Deglaciation and recovery of primary production was part of a feedback loop, which resulted in the reestablishment of strong euxinic oceanic conditions [69,70]. This was the result of extensive continental weathering following the accumulation of atmospheric CO_2 during the glacial period [69]. A large flux of CH_4 into the atmosphere played an important role in deglaciation [69] from active methanogenesis that may have utilized methyl sulfide compounds from seawater [69,70], volcanic activity [227], and the release of methane from methane clathrates [69,228]. Though still debated, methanogenesis likely played a key role in the formation of these methane clathrates [228]. Methanogens that produce CH_4 under low temperature and/or high pressure are known to contribute to clathrate formation [229]. The production and consecutive release of methane from clathrates to the atmosphere has also been invoked later in the more recent events like the melting of the Laurentide–Cordilleran ice sheet [230].

2.2.4. Methanogens Through Mass Extinctions

Widespread and regional anoxic and euxinic ocean conditions have been characteristic of multiple extinction events , such as those found in Western Tethys and Panthalassa at the end Triassic mass extinction (~201.6 Mya) [206,231] and during the Permian mass extinction (252Mya) [232]. A rise in global temperature following rapid release of greenhouse gases (CO_2 and CH_4) [71,206,231], via volcanism (e.g., the Siberian Traps) [233], destabilization of methane reservoirs (e.g., methane clathrates/hydrates and permafrost) [206,232], and/or microbial sources (i.e., methanogenesis) [206]. These anoxic reservoirs could have fueled methanogen activity, creating a positive feedback loop that further contributed to a global rise in temperature, which in turn led to melting and release of additional methane. The End Triassic mass extinction experienced increases in acetate and H_2 availability and a decrease in ocean sulfate concentrations that may have contributed to increased methane flux from methanogen activity in the oceans [206]. Likewise, following the Permian mass extinction, a euxinic photic zone and low ocean oxygen concentrations led to a buildup of organic material in sediments [234]. The degradation of this organic matter was likely both a substrate for methanogenesis and a source of acetate, which could have supported the evolution of the modern-day high efficiency aceticlastic pathway [234] in *Methanoscarcina spp*. [235]. Increased CH_4 and H_2S fluxes from the oceans during the Permian reduced atmospheric O_2 and O_3 by ~14% and consequently decreased CH_4 oxidation in the atmosphere [206]. Under lower atmospheric O_2 conditions like this, aerobic and anaerobic methane oxidation may play a critical role in the removal of CH_4, in environments with and without oxygen, respectively [236]. Similar conditions may have been present during other mass extinction events [237], even though methanogenic activity during these times is not discussed extensively in the literature.

2.2.4.1. Resurgence of Methanogens and Microbialites Following Mass Extinctions

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Methanogens often thrive following mass extinction events, significantly delaying ecosystem recovery, notably in marine environments. By disrupting the global carbon cycle, methanogens can create persistent toxic conditions, resulting in "dead zones". Consequently, re-establishment of complex life is difficult due to warming and anoxic effects due to CH_4, slowing the return of diverse ecosystems [238].

There is often an increase in the abundance of microbialites following mass extinction events [239] due to a carbonate-saturated, alkaline ocean, increased availability of nutrients (e.g., nitrogen-fixing cyanobacteria, weathering) [240,241], and a lack of competition with decimated eukaryote populations (i.e., decreased bioturbation and grazing pressure) [239,242,243]. Following the Permian-Triassic extinction event, the abundance of organic carbon combined with dysoxic conditions likely supported sulfate-reduction [244] and methanogenesis [234], and possibly anoxygenic photosynthesis by *Gakhumella*-like cyanobacteria [245]. However, early Triassic oceans may have had a low oceanic sulfate concentration [246], which would have profited methanogens [234]. Importantly, all three anaerobic metabolisms favor lithification of mats, potentially forming stromatolites [4,196]. A similar resurgence of microbialites under anoxic or dysoxic conditions, possibly supported in part by methanogens, occurred, e.g., during the Early Silurian, following the Late Ordovician Extinction Event [247] and the end-Devonian mass extinction [248]

2.3. Past, Present, and Future – Methanogens in Climate Change

Methanogens still play an essential role in the modern-day climate, producing over 70% of biogenic methane emissions annually [249,250]. Although methane has a lower abundance in the atmosphere (~1.4 to 1.9 ppm; [48,251] than CO_2, at ~360 to 390 ppm [252,253], it is about 30 times more potent as a greenhouse gas [3]. Methanogens process in excess of 500 Tg C y^{-1} through their metabolism in the production of CH_4 and biomass [254]. However, methanogens may play an additional role in the global carbon cycle by mediating precipitation and dissolution of carbonate minerals [255], thus impacting long-term storage of atmospheric CO_2 (**Figure 2**) [162]. This makes their specific role in the carbon cycle more complex than previously thought.

Lithotrophic metabolisms are believed to promote the precipitation of carbonate minerals [255] (Equation (3)) where removal of CO_2 favors the production of CO_3^{2-} ions, increasing pH (Equation (9)) in the surrounding environment. This could promote the precipitation of carbonates, such as $CaCO_3$ [4,196] (Equation (10), *net* Equation (11)).

$$HCO_3^- \rightarrow CO_2 + OH^- \quad (9)$$

$$HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+ \textit{ and } H^+ + OH^- \rightarrow H_2O \quad (10)$$

$$2HCO_3^- + 4H_2 + Ca^{2+} \rightarrow CaCO_3 + CH_4 + 3H_2O \quad (11)$$

However, other types of methanogenesis have different effects on the $CaCO_3$ budget. Methanogens produce exoplymeric substance (EPS) [256], which could also aid in the process of mineralization [196,217]. On the other hand, aceticlastic (Equation (1)) and methylotrophic (Equation (2)) species may promote the dissolution of $CaCO_3$ minerals through a decrease in pH, where CO_2 is produced as a product of the metabolisms (Equations (1) and (2)). The increase in pH would favor HCO_3^- ions (Equation (12)) and promote the dissolution of $CaCO_3$ minerals (Equation (13); *net (for aceticlastic methanogenesis)* Equation (14)). In which case, carbonate minerals could serve as a potential carbon source for methanogen growth [162].

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$$CO_2 + OH^- \rightarrow HCO_3^- \quad (12)$$

$$H_2O \rightarrow H^+ + OH^- \textit{ and } CaCO_3 + H^+ \rightarrow HCO_3^- + Ca^{2+} \quad (13)$$

$$CH_3COOH + CaCO_3 + H_2 \rightarrow 2HCO_3^- + CH_4 + Ca^{2+} \quad (14)$$

This dual role that methanogens play in the global carbon cycle and their metabolic and biogeochemical role in $CaCO_3$ production remains unstudied, leaving gaps in our understanding of the capacity of microbes to regulate planetary climates. Currently, studies of methanogen-driven carbonate mineralization are limited to wastewater [256–258], carbonates in authigenic marine sediments [259,260], and Mn carbonates formed as a product of the methanogenic pathway [261]. Methanogens are abundant in many sediments, including microbial mats [24,43], which makes mat ecosystems prime locations to study methanogenic $CaCO_3$ precipitation [42,262]. Methanogens occupy a unique niche within mats, relying mostly on non-competitive methylated compounds for growth, such as methylamines and methylsulfides are not used by SRB [43,263,264].

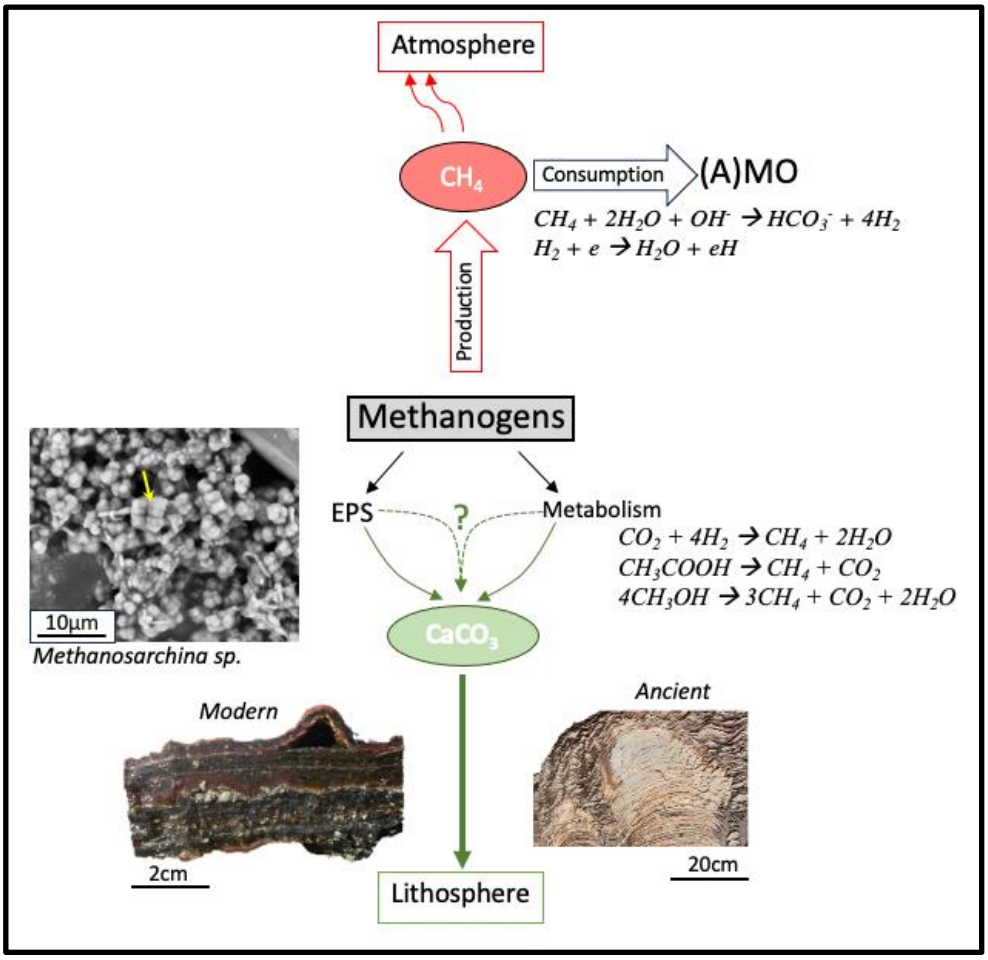

Figure 2. The multiple roles of methanogens in the modern-day carbon cycle. Processes shown in red contribute to greenhouse gas production. Part of the methane is removed through microbial methane oxidation (A)MO and photochemical reactions, where *e* is a generic electron donor (e.g., arsenate or sulfate) that is reduced to *eH*. Processes depicted in green sequester CO_2, storing it long-term as carbonates. The size of the arrows relates to the relative biogeochemical importance of the process. The metabolic activity of methanogens may locally alter pH and produce copious amounts of EPS (seen as a veil surrounding the cells in the microphotograph (yellow

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arrow)). These EPS bind cations (e.g., Ca, Mg) and act as nucleation site, while elevated pH enhances the carbonate precipitation in microbial mats [196]. This contribution by methanogens may ultimately produce microbialites [183].

3. Methanogens in Microbial Mats as Biosignatures for Life

Organisms in microbial mats must cope with a range of not only extreme, but also fluctuating conditions [4]. Sulfide and oxygen gradients in these systems fluctuate over diel cycles [4,196,217], with O_2 peaking during the early afternoon, along with peak photosynthesis by cyanobacteria, reaching daily peaks of > 600% O_2 saturation in lithifying mats [4,60]. In contrast, peak sulfide concentrations are found near the mat surface, peaking at the end of the night, associated with sulfate reduction activity [4,196].

While the modern-day diversity of archaea in microbial mats is limited [262,265], methanogens have been identified (via their metabolic activity) in the anoxic but also the oxic zone of microbial mats [24,42,43,266,267]. Microbial mats are often found in extreme environments; in hypersaline conditions, when the salinity exceeds 180 PSU, methanogens can outcompete other anaerobic heterotrophs, notably SRB [60]. Desiccation impacts microbial mat communities through wetting and drying cycles [50] that enhance compatible solute production. As noted above, several of these compounds are precursors to methylamines and methylsulfides used by methanogens [42,267]. Desiccation has been proposed as a survival strategy in methanogens [50]. These authors reported that the viability of methanosarcinales was greatly enhanced by EPS, and that desiccated organisms had a higher resistance to high temperature and oxidative conditions. These properties may prove useful in extraterrestrial environments, such as Mars (*See Section 4*) [37].

3.1. The Evolution of Microbial Mats and Stromatolites

Microbial mats are organosedimentary systems made up of complex microbial communities embedded in an exopolymeric matrix [217]. Some mat communities facilitate microbially induced $CaCO_3$ precipitation and produce lithifying mats [4,196,255]. The precipitation potential is determined by the combined metabolic activities of the entire microbial community and by the environmental (physicochemical) conditions [4,268]. Complete element cycling, notably of C, O, and S, is very efficient in these semi-closed, self-sustaining [255]. Microbial mats could have been among the first complex microbial communities to evolve on Earth [212], in shallow ponds near hot springs [183,269], appearing in the fossil record as stromatolites as early as ~3.5-3.7 Gya [220,222]. A variety of available chemical elements supporting a range of metabolisms, combined with a close proximity of an assortment of microorganisms [270] characteristic of mats, could have been instrumental in the diversification of microbial life.

Both lithified and non-lithified (soft) mats biofilm systems were more abundant during the Archean and Proterozoic eons, but in modern times have been forced to environments with extreme conditions likely due to competition with eukaryotes [4,271]. The presence of methanogenic activity in the oldest microbial mats and stromatolites has been suggested, mainly based on carbon isotopic evidence and metal availability[62,183,272,273]. Methanogens are still involved in the formation of modern microbialites [255], although actual their role in the lithification process remains largely unknown.

3.2. The Conundrum of Methanogenesis and Oxygen in Mats

It is yet unclear how methanogens cope with the fluctuating conditions in microbial mats. Notably, the presence of supersaturated oxygen concentrations during the early afternoon creates a challenge for these anaerobic organisms [24] due to the oxygen-sensitivity of methyl-coenzyme M reductase [274]. Although methanogens possess biochemical properties, such as ether-linked membrane lipids [275] or pseudomurein cell walls or proteinaceous sheaths [276], that make these microbes more resistant to extreme conditions (e.g., pH, temperature, mechanical stress, salinity),

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other survival mechanisms are employed. Methanogens do not form endospores but survive in a dormant state followed by rapid recovery [277]. Such a state of reduced activity is accomplished during desiccation. Additionally, there are specific solutions to oxygen-related stress: although glutathione has not been found in methanogens, enzymes like superoxide dismutase, catalase, and peroxidase cope directly with reactive oxygen species, and repair mechanisms of oxygen damage (e.g., disulfide reductases) also exist [102,278]. Other physiological adaptations include modified electron transport chains, production of protein-rich EPS, formation of cell clusters, polyphosphate accumulation, or a metabolic shift to methylated substrate use [50,102,276]. Quorum sensing has been reported in some methanogens[102,124], which hypothetically could allow for cyanobacteria, methanotrophs, and methanogens to live in close proximity while metabolically active (**Figure 3**). Such a model was proposed previously for the coexistence of sulfate-reducers, sulfide-oxidizers, and cyanobacteria [279]. Last but not least, other ecological adaptations may enable methanogens to thrive in oxic conditions. These include formation of consortia [280], syntrophism [281], and interspecies transfer of electrons [282].

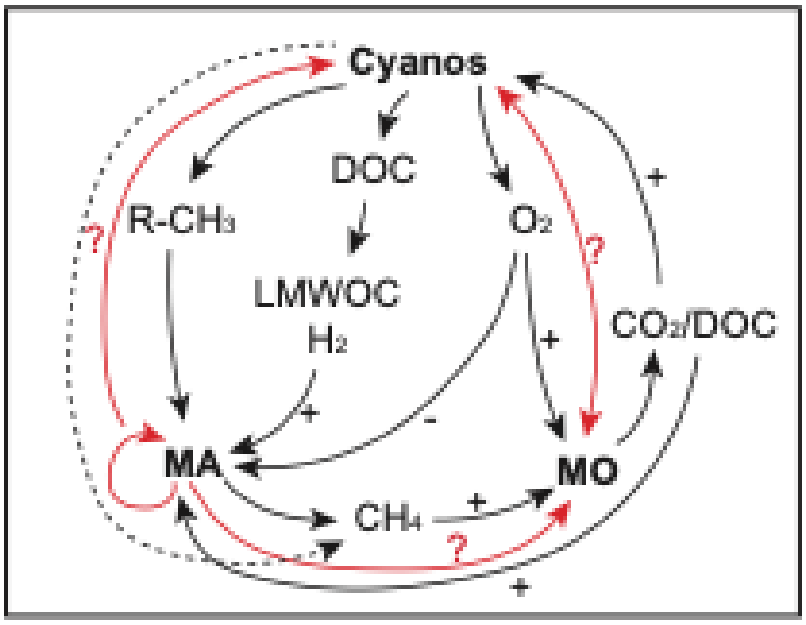

Figure 3. Hypothetical interactions between cyanobacteria, methanogens and methane oxidizers in the oxic layer of a microbial mat. Quorum sensing (red arrows) between consortia of cyanobacteria, sulfate reducers, and sulfide-oxidizing bacteria was proposed to allow for maximum metabolic potential under unfavorable conditions [279]. Methanogens (MA) may facilitate quorum sensing in a similar manner between methane oxidizers (MO), and/or cyanobacteria [4]. Modified from [279].

4. Methanogens in the Search for Extraterrestrial Life

Methanogens are of prime interest for possible life on exoplanets. The primary product of their metabolism, methane, is an ideal gas for detection in remote atmospheres due to the unique absorption spectrum and short photochemical lifespan [6]. To date, methane has been detected on several planetary bodies in our solar system that may be fit for supporting methanogenesis-like life, including Mars [283], Saturn's moon Enceladus [284], and Titan [285]. The detection of methane on other planets and its short residency time in planetary atmospheres suggests sources for an active methane flux on these planetary bodies, which on Earth, primarily is of biological origin [6]. The potential of methane, through the greenhouse effect, to warm planetary atmospheres may expand the possibility for life beyond the "Goldilocks" zone [10].

Many studies have assessed methanogen survival under Mars-like conditions [30,36,37,39,286] and their potential habitability on ice worlds like Enceladus [13]. The extreme environments many

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methanogens are found are used as analogs for extraterrestrial bodies [10,35,168]. For example, soda lakes are studied for their potential as analogs to Mars's ancient ocean [35,287]. Similarly, hydrothermal vents are expected to exist on other worlds, like Enceladus, where products of serpentinization reactions and silica-rich particles have been detected [13]. Thermophilic methanogens may be able to survive at hydrothermal vent sites on Enceladus, and the predicted temperature and pressure associated with this ecological extreme [13]. Survival of methanogens to conditions on Mars (e.g., low pressure at the surface (6 mbar), desiccated soil, high UV radiation at the surface, and freezing surface conditions and permafrost due to lack of an atmosphere) [30,36,37,39,78,286] has supported the idea of potential subsurface life on the planet [36], or more broadly, a subsurface source for methane [288]. Microbial mats themselves are useful biosignatures due to their self-sustainability, resistance to extremes, and abundance on early Earth [168].

5. Conclusions

Methanogens were among the earliest microorganisms to evolve on Earth and continue to thrive in a wide range of both seemingly 'non-extreme' and 'extreme' environments today, several of which are similar to those throughout Earth's history (**Figure 4**). Microbial mats are a prime example of ecosystems that evolved with our planet (or, arguably, changed our planet's properties) and were present in abundance during the Archean and Proterozoic eons. Methanogens were likely part of the early stromatolite communities and continued to be ubiquitous in microbial mat ecosystems through time [212]. The transition from strictly oxic conditions during the day and anoxic ones during the night may have supported methanogens to be well-adapted to a wide range of sometimes fluctuating conditions (e.g., diel fluctuations of O_2, sulfide, pH, and light gradients) [4]. The lack of a proper understanding of the ecophysiological capabilities and flexibilities (e.g., EPS properties, stress responses, viral-host relationships, cell-to-cell communication) is a major gap in the understanding of the role and evolution of methanogens and their impact on our planet through geologic time. Their elementary metabolism (e.g., respiration of H_2/CO_2), efficient use of a minimal energy yield, and the simplicity of their main metabolic product (i.e., CH_4) – which is observable remotely and supports a diversity of photochemical reactions – make methanogens the ultimate candidates for astrobiological studies.

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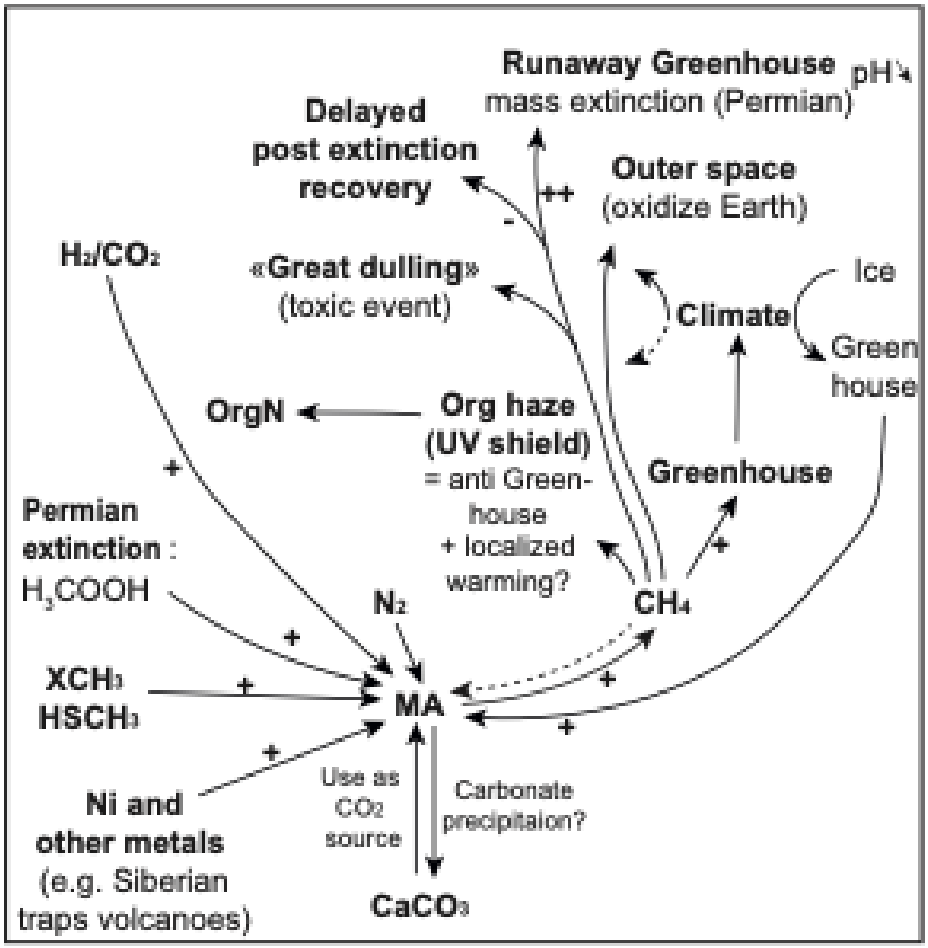

Figure 4. Summary of the various roles of methanogens in biogeochemical processes, including positive and negative feedback, through geologic time that impacted climate. The major processes methanogens participate in include: i) consumption of organic carbon (e.g., XCH_3, or methylated compounds), producing biomass and CH_4 and fractionating stable isotopes of carbon; ii) consumption of H_2/CO_2 (e.g., from Earth's early atmosphere), potentially changing the redox balance; iii) precipitation of minerals (e.g., carbonates); iv) use and fractionation of trace elements (e.g., Ni) that stimulated their growth and metabolic activity; v) the release of CH_4 to the environment, stimulating methane-consuming microbes; vi) the release of CH_4 to the atmosphere, acting as a greenhouse gas, contributing to organic haze formation that yield organic nitrogen, thereby regulating climate and consequently, play a role in beginning and/or end of mass extinctions and the ecological recovery after these icehouse events. Note that the strong isotopic fractionation of carbon and nickel by methanogens can be used to reconstruct early ecosystems on Earth. The production of methane and fractionation of carbon is a potential biosignature that can be used in the search for life beyond our planet.

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