1 FINAL REVISED VERSION

3 Geologic hydrogen and methane as fuel for life

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9 Title Image

12 Confocal laser scanning microscopy image of microbial filaments inhabiting juvenile chimneys of
13 the serpentinization-related hydrothermal field of the Prony bay (New Caledonia). These
14 filaments (highlighted in green by a fluorescent DNA probe) are mineralized by brucite
15 ($\text{Mg}(\text{OH})_2$, in blue) and are likely utilizing serpentinization-derived organic compounds (Pisapia
16 et al. 2017). Courtesy of Emmanuelle Gérard (Université de Paris, Institut de physique du globe
17 de Paris, France).

19 ABSTRACT

20 Geologically-produced molecular hydrogen and methane could be widely utilized by
21 microbial communities in surface and subsurface environments, that potentially impact

the net emissions of H₂ and CH₄ to Earth's ocean and atmosphere. Geologic H₂ and CH₄ could enable microbial communities to exist in rock-hosted environments and hydrothermal systems with little or no input from photosynthetic carbon fixation, making these communities potential analogs for the earliest metabolisms on Earth and other planetary bodies. The possible dependence of rock-hosted ecosystems on H₂ and CH₄ also must figure in current and future plans for engineering the subsurface for storage of these compounds as energy fuels.

KEYWORDS: deep biosphere, biological hydrogen utilization and production; abiotic organic compounds, subsurface storage, early metabolisms

INTRODUCTION

The possibility that reduced compounds such as molecular hydrogen (H₂; Klein et al. 2020 this issue) and methane (CH₄; Reeves and Fiebig 2020 this issue) form through geologic processes (e.g., water/rock interaction, water radiolysis, rock fracturing) has strong implications for microbial life throughout Earth history. Molecular hydrogen and hydrocarbons such as CH₄ have the potential to support chemosynthetic populations that may use these compounds to fuel their metabolisms and to generate biomass. Such microbial communities may exist within geologic formations up to several kilometers deep, as long as temperature (<122°C), water and nutrient requirements are met. Life in these environments might be disconnected from photosynthesis and totally rely on the geosphere for energy and carbon supply. Considering that the vast majority of microorganisms on Earth live in the subsurface (e.g., Magnabosco et al. 2018), abiotic H₂ and CH₄ may constitute critical electron donors and carbon sources for the deep biosphere and more broadly, for life on our planet. However, the extent to which the deep biosphere is supported by geologic H₂ and CH₄ remains an open question. Moreover, key questions remain regarding the composition of abiotic organic compounds available at depth and their respective utilization by chemosynthetic microbial communities. Low molecular-weight organic acids of abiotic origin, such as formate (HCOO⁻) and acetate (CH₃COO⁻), have been shown to play an important role in supporting communities inhabiting subsurface environments (e.g., Lang et al. 2018, Fones et al. 2019). Recent evidence for abiotic amino acids and carbonaceous matter in habitable rocky environments suggests the diversity of abiotic organic compounds to be

higher than previously thought, although their bioavailability remains to be assessed (e.g., Andreani and Ménez 2019).

Hydrothermal systems driven by water circulation through the oceanic lithosphere are the most intensively studied ecosystems with evidence indicating that their microbial inhabitants are sustained by H_2 , CH_4 , formate, and acetate (e.g., Lang et al. 2018). Such work includes submarine hydrothermal fields along mid-ocean ridges comprising high temperature and acidic basalt-hosted vents, as well as alkaline and warm serpentinization-related vents hosted on mantle-derived rocks (e.g., Adam and Perner 2018). Hydrogen- and methane-dependent microorganisms also dominate in groundwater circulating in ophiolites (e.g., Suzuki et al. 2014, Brazelton et al. 2017, Miller et al. 2016;) and in mud volcanoes formed in subduction zones (e.g., Trembath-Reichert et al. 2019), two environments where active serpentinization also occurs. Microbial communities relying on a geologic supply of H_2 also have been identified in Precambrian cratons where ancient waters trapped in deep fractures undergo radiolysis during radioactive decay of U, Th, and K in the host rock, producing H_2 and activating the abiotic synthesis of organic compounds (e.g., Li et al. 2016). Chemical reactions between water and freshly broken mineral surfaces can also support episodic or slowly-sustained production of H_2 in a diversity of geological settings undergoing rock fracturing and crushing, including faults or beneath sliding glaciers (e.g., Telling et al. 2015). These H_2 fluxes may support H_2 -utilizing communities including methanogens in a large variety of rocks, as experimentally demonstrated for granite (Parkes et al. 2018).

In this article we first review how recent progress in microbial ecology significantly increased our knowledge regarding the microbial utilization of H_2 and CH_4 . We summarize the reasons why H_2 metabolisms are widespread in a large variety of environments. In almost every system where H_2 and CH_4 are geologically formed, we review how these compounds are both biologically consumed and produced, which complicates the assessment of the origin of H_2 and CH_4 as well as the overall influence of H_2 - and CH_4 -dependent metabolisms on associated global biogeochemical cycles. We then consider the implications for subsurface engineering such as carbon capture and storage or the underground storage of H_2 and CH_4 as energy fuels. Finally, we discuss the potential role of geologic H_2 and CH_4 in the earliest metabolisms on Earth.

89 FROM CULTIVATION-BASED APPROACHES TO METAGENOMIC SURVEYS

90 The capability of microbes to rely on H₂ or CH₄ as an electron donor (or alternatively, to
91 produce these compounds through their metabolism) can be investigated by isolating
92 species using enrichment cultivation techniques, and by measuring consumption and
93 production of metabolites. This method represents the oldest and only approach
94 available before the rise of molecular biology methods that target biological
95 macromolecules such as nucleic acids or proteins. Pure cultures make it possible to
96 characterize the physiology of H₂- or CH₄-metabolizing microorganisms, which in turn
97 can inform on their ecology. Experimental work with pure cultures or enriched
98 consortia also allows for the development of proxies of H₂- and CH₄-related microbial
99 activity in environmental settings. For example, deuterium (δD) and carbon ($\delta^{13}C$) stable
100 isotope compositions are commonly used to distinguish abiotic and biologically
101 produced CH₄ (e.g., Etiope and Sherwood-Lollar 2013). Deuterium depletion in fatty
102 acids also can be a signature of H₂-metabolizing bacteria (e.g., Campbell et al. 2009).
103 However, the difficulty to reproduce appropriate growth conditions combined with the
104 low growth rates and the low microbial cell density typifying subsurface environments
105 (frequently well below 10⁵ cells/ml in groundwaters related to serpentinizing systems –
106 e.g., Brazelton et al. 2017, Fones et al. 2019 – and as low as 10³ cells/gram or less in
107 oceanic rocks – Quéméneur et al. 2019), has led to very few environmentally-relevant
108 isolated representatives (e.g., Suzuki et al. 2014, Quéméneur et al. 2019 and references
109 therein for serpentinization-related environments). Thus, most microbes from these
110 subsurface and extreme environments remain uncultured.

111

112 The development of cultivation-independent approaches based on the sequencing and
113 analysis of environmental DNA has allowed a better assessment of the diversity of
114 microorganisms involved in H₂ and CH₄ cycling. These approaches were first based on
115 the analysis of a marker gene, which can either be a taxonomically and phylogenetically
116 informative gene such as a part of the gene encoding the small subunit (16S) of
117 ribosomal RNA (rRNA) that is widely distributed among Bacteria and Archaea. This
118 makes it possible to describe the diversity of microorganisms present (i.e., “who is
119 there?”) and to estimate their relative abundance in the community. Alternatively,
120 functional genes involved in a specific metabolic pathway can be targeted as markers to

describe the potential for a given biogeochemical process to be taking place, and to identify what organism is likely responsible for the activity (i.e., “what are they capable of doing?”).

As an illustration, microbial H₂-cycling involves the use of hydrogenase proteins, which are specialized metalloenzymes that can be used either in H₂ uptake and oxidation (i.e., hydrogenotrophy) or in microbial H₂ production (i.e., hydrogenesis). Hydrogenases catalyze the reversible oxidation of H₂ to yield protons and electrons through the reaction $\text{H}_2 \leftrightarrow 2\text{H}^+ + 2\text{e}^-$. They are classified into three main phylogenetically-unrelated classes based on their amino acid sequence, metal content and functionality: the [NiFe]-, [FeFe]- and [Fe]-hydrogenases. [NiFe]-hydrogenases can be broken down into several different phylogenetic subclasses that are predictive of their directionality (i.e., H₂ oxidation or/and H₂ production; e.g., Greening et al. 2016, Adam and Perner 2018). Likewise, specific amino acids near the active site of [FeFe]-hydrogenases can, in some cases, be used to infer the ratio between reaction rates for H₂ oxidation and H₂ production. Hence amplification and/or sequencing of hydrogenase encoding genes from environmental DNA, when placed within a phylogenetic and bioinformatics framework, allow for the characterization of the potential for H₂ oxidation or production in a community (e.g., Greening et al. 2016, Lindsay et al. 2019).

Similarly, genes encoding proteins involved in microbial CH₄ production or consumption can be used to gauge the potential for these processes in a natural microbial community. Methanotrophy, or the oxidation of CH₄ for use as a source of carbon and energy, can be detected using the gene encoding the alpha subunit of methane monooxygenases (MMOs). The gene encoding the alpha subunit of the methyl coenzyme M reductase (*mcrA*) is predominantly used as the functional marker for microbial methanogenesis, i.e., the production of CH₄ from, for example, CO₂, methanol CH₃OH, formate and acetate. Importantly, some anaerobic CH₄-oxidizing Archaea also use this enzyme, which can nonetheless be readily differentiated from those involved in CH₄ production using evolutionary analysis. Analysis of marker genes then allows one to infer biological functions and to establish relationships between taxonomy and functions. However, the utility of these approaches strongly depends on how well the genomic diversity of a

given community is represented in sequence databases, which can be critical for the characterization of poorly studied deep and extreme environments.

In the past 15 years, the increase in the throughput of metagenomic sequencing and associated computational approaches, combined with a significant reduction in cost, has allowed progress in characterizing the taxonomic and functional diversity of microbial communities. Metagenomics — “meta” meaning “beyond” to refer to mixed populations rather than single organisms, and “genomics” meaning the study of genomes — is based on the shotgun sequencing of the total DNA extracted from environmental communities. Instead of targeting only specific marker genes, it provides broader insight into the diversity and functional potential of the populations that comprise a microbial community. As illustrated in FIG. 1 (Sharpton 2014), it is achieved by computationally assembling small DNA fragments (i.e., the sequenced ‘reads’) into larger sequences (i.e., the ‘contigs’) that can then be binned into metagenome assembled genomes (MAGs). This is followed by analyses of the taxonomy and function of genes encoded in either the ‘contigs’ or the MAGs using bioinformatics approaches. Several thousands of genomes of uncultivated Bacteria and Archaea, many from new phyla lacking isolated representatives in laboratory cultures, have been assembled over the past several years. These genomic data have substantially expanded our understanding of microbial diversity and associated metabolic capabilities, how communities are structured, how inter-organism interactions shape ecosystem function, and how microbial diversity associates with and scales across environmental conditions. As an illustration, such an approach was key to ascribing an important role to H₂ in supporting serpentinite-hosted microbial communities (e.g., Adam and Perner 2018 and reference therein) and to suggesting a role for CH₄ cycling in these populations (e.g., Brazelton et al. 2017). Importantly, gene surveys of metagenomes enable exploration of the functional potential of a community but does not guarantee that microorganisms are actively carrying out these functions. To assess if genes were expressed at the time of sampling, metagenomics can be paired with sequencing of messenger RNA (i.e., metatranscriptomics) or proteins (i.e., metaproteomics)(e.g., Lindsay et al. 2019).

H₂ AS A KEY DRIVER FOR LIFE

Molecular hydrogen represents a reductant capable of supporting a diversity of microbial forms of life. Due to its reduction potential and its ability to diffuse across biological membranes, H₂ is an invaluable electron donor for microbial metabolisms. Splitting of H₂ into electrons and protons also allows the generation of proton gradients in cells and hence the synthesis of adenosine triphosphate (ATP), which provides energy to drive many processes in living organisms, including autotrophic CO₂ fixation. Conversely, during fermentation and photobiological pathways, the high diffusivity of H₂ allows efficient dissipation of excess reductants from the cell, hence making H₂ a common byproduct in these processes.

Contrary to what has long been believed, H₂-based microbial metabolisms are not restricted to specific groups of organisms inhabiting certain types of environments. Instead, as demonstrated by recent physiological and ecological studies, H₂ is widely used among Bacteria, Archaea and to a lesser extent Eukaryotes from a wide range of environments (e.g., Peters et al. 2015, Greening et al. 2016). Hydrogen oxidation can be coupled with the reduction of numerous electron acceptors including CO₂ or HCO₃⁻ from carbonate minerals, sulfate, nitrate, and ferric iron from iron oxides, as well as strong oxidants such as O₂ (e.g., Adam and Perner 2018). Hence, molecular hydrogen is capable of fueling the energy metabolism of populations inhabiting both anoxic and oxic ecosystems. The large diversity of biochemically distinct hydrogenases with different affinity for H₂ and different sensitivity to O₂ (Peters et al. 2015) allows this reductant to be used by taxa inhabiting various temperature regimes and environmental conditions, including high pH and salinity. As recently reviewed in Adam and Perner (2018), diverse H₂ oxidizers have been isolated from thermally and chemically distinct hydrothermal vents. Similarly, hyperalkaline springs in ophiolites contain numerous H₂ oxidizers including the recently isolated, proposed genus *Serpentinomonas* (e.g., Suzuki et al. 2014).

As illustrated in FIG. 2, a recent survey of hydrogenase encoding genes of metagenomes retrieved from various surface environments shows the presence of diverse hydrogenase sequences and, by extension, a diverse suite of taxa (representing 51 different phyla) that likely rely on H₂ in respiration, fermentation and carbon fixation processes (Greening et al. 2016). It comprises hundreds of bacterial species belonging to

Proteobacteria, Firmicutes and Actinobacteria and tens of archaeal species from the Euryarchaeota and Crenarchaeota phyla (FIG. 2A). Interestingly, these phyla are well represented in subsurface and hydrothermal ecosystems relying on geochemical energy sources, suggesting that they may play an important role in H₂ consumption or production in these geologic settings (e.g., Adam and Perner 2018). FIG. 2B shows that although hydrogenases are widely distributed in surface environments, they are mainly found in hot springs in addition to soils. In subsurface environments, the abundances of hydrogenases were estimated to be nearly one order of magnitude higher compared to surface environments (Colman et al. 2017). The potentials of subterranean environments and hydrothermal systems for sustained fluxes of abiotic H₂ ($\pm$ organic compounds) through deep crustal outgassing, crystallization of basaltic lava, water/rock interactions, water radiolysis or rock fracturing and crushing (Klein et al. 2020 this issue) may explain such a predominance of this metabolic lifestyle. As illustrated in FIG. 3, abiotic H₂ and organic compounds may therefore underlie deep ecosystems, as supported by the recognition of the large contribution of H₂ metabolisms to total ecosystem processes associated with hydrothermal and subsurface environments (e.g., Colman et al. 2017, Adam and Perner 2018).

MICROBIAL UTILIZATION OF ABIOTIC H₂ AND CH₄

FIG. 3 illustrates the diversity of the metabolic pathways potentially cycling H₂ and CH₄ in (deep) microbial ecosystems, showing that abiotic H₂ and CH₄ are both valuable electron donors and carbon sources for chemolithotrophic ecosystems functioning with little or no input from photosynthesis. In addition to aerobes oxidizing H₂, abiotic H₂ can fuel autotrophic acetogens and methanogens as well as chemolithotrophs reducing oxidized sulfur species such as sulfate, oxidized nitrogen species such as nitrate or iron (and manganese) oxides. Abiotic CH₄ can sustain anaerobic and aerobic methanotrophs, again using oxidized sulfur and nitrogen species, iron (and manganese) oxides or O₂ as electron acceptors. FIG. 3 also highlights the capability of microbial populations to use other geologically-produced organic compounds such as formate, acetate, methanol (CH₃OH) and polycyclic aromatic hydrocarbon (PAHs) as their electron donors instead of H₂ or CH₄ (e.g., Trias et al. 2017, Lang et al. 2018, Fones et al. 2019). Among the latter, formate, whose formation is favored during serpentinization at high H₂ levels, is the only one for which a conclusive abiotic origin has been demonstrated in natural

environments (Lang et al. 2018), although the abiotic formation of acetate, methanol and PAHs is supported by experiments and/or thermodynamic calculations.

The potential of all these metabolisms to act as primary producers for an entire microbial community is supported by thermodynamic calculations (e.g., Reveillaud et al. 2016). Such calculations allow for an estimation of the metabolic energy — i.e., the energy required to run the anabolic and catabolic reactions that allow organisms to live and grow — that derives from a naturally occurring process and to provide predictions on electron donor/acceptor pairs that yield the most energy in a given geologic setting. As an illustration, FIG. 4 shows the catabolic energy available in oceanic vents where hydrothermal fluids mix with oxidized seawater. It highlights that, compared to basalts, peridotite in contact with oxidized fluids can provide a more energetically favorable landscape for sustaining chemolithotrophic life, including H₂-driven sulfate reduction and methanogenesis, as well as anaerobic CH₄ oxidation (Reveillaud et al. 2016). The higher energy available in hydrothermal fluids derived from the alteration of peridotites is primarily due to higher abundance of abiotic H₂ and CH₄ typically associated with serpentinization-derived fluids vs. fluids from altered basalts. Accordingly, the level at which abiotic H₂- and CH₄-cycling microbes are present and active in a given environment strongly depends on the geologic context and the energy sources provided by the host rock lithologies. This alone emphasizes the driving role of the rocky environment in shaping microbial communities. Nevertheless, the host rock also imposes potentially challenging conditions in term of pH, availability of electron acceptors, and carbon sources that also depend on the extent of fluid mixing and water/rock interactions. It implies the need for physiological adaptations for microbes to persist under these poly-extreme conditions and to take advantage of the abiotically produced energy and carbon sources (e.g., Fones et al. 2019). The abundances, taxonomy and diversity of microbial cells, their functional potential, and their rates of substrate utilization, including abiotic H₂ and CH₄, hence strongly depend on the host rock and the extent of interactions with external fluids.

Marine hydrothermal systems are some of the largest reservoirs of H₂-utilizing microorganisms (Adam and Perner 2018), but H₂ also has been shown to shape and fuel microbial communities in more diverse subsurface environments including continental

aquifers. One of the most striking examples of the biological utilization of abiotic H₂ can be found in the reduced fracture fluids sampled in deep subterranean environments such as the South African gold mines. The low-diversity microbial community sampled in these fluids at 2.8 to 4.2 km below the Earth's surface is dominated by *Desulforudis audaxviator*, a Firmicute able to thrive through hydrogenotrophy coupled to sulfate reduction (e.g. Chivian et al. 2008). This latter case study has similar geologic and geochemical characteristics to deep mines located in the Precambrian Canadian shield (Li et al. 2016). Water radiolysis, induced by the radioactive decay of natural radionuclides in the host rock, provides disequilibrium in redox reactions to sustain microbial metabolisms in these systems. Radiolysis drives the abiotic production of H₂, as well as the generation of reactive oxygen species potentially involved in the formation of sulfate from the oxidation of hydrothermal iron sulfides (Li et al. 2016). Although Chivian et al. (2008) show that *D. audaxviator* is able to thrive almost alone (constituting 99.9% of the microbial community) from H₂ in some of these deep fractures, this Firmicute is found associated with methanogens in other fracture fluids of the same mine. H₂-utilizing Betaproteobacteria such as Burkholderiales are also frequently associated with methanogens in various geologic settings worldwide. Such an association points to potential competition for abiotic H₂ between autotrophic methanogens and sulfate reducers or alternatively, establishment of symbiotic relationships (e.g., Lang et al. 2018; Ranchou-Peyruse et al. 2019).

DECIPHERING THE MICROBIAL IMPRINT ON NATURAL H₂ AND CH₄ FLUXES

Abiotic H₂ and CH₄ are not only important as reductants for microbial life, they also are metabolic byproducts (FIG. 3). Such biologically-produced H₂ or CH₄ can be used by other members of the microbial community or can be released from the ecosystem, contributing to the overall fluxes of H₂ and CH₄ detected at the Earth's surface.

As mentioned earlier, H₂ can be a major byproduct of fermentation of organic compounds, which is an anoxic process occurring when available electron acceptors become depleted, as is frequently the case in the anoxic subsurface. In the serpentinizing subsurface, fermentation also could be promoted by abiotic organic molecules that are expected to be found in higher abundance compared to inorganic carbon species. Abiotic amino or condensed carbonaceous matter acids are other possible yet unproved energy

sources for fermentation (e.g. Andreani and Ménez 2019) (Andreani and Ménez, 2019). The recent finding of these potential substrates in serpentinizing environments suggests they may be feedstocks for multiple populations, with the potential to impact natural H₂ and CH₄ budgets. Hydrogen production was demonstrated for fermentative Firmicutes isolated from active alkaline vents in ophiolites and the Firmicute *Petrocella atlantisensis*, which is the first bacterium isolated from the oceanic crystalline basement, only grows by fermentation of sugars and amino acids, forming acetate, formate, H₂ and CO₂ (Quéméneur et al. 2019 and references therein). Biologically-produced H₂ also can derive from aerobic and anaerobic nitrogen fixation and anaerobic carbon monoxide (CO) oxidation, which are common metabolisms in the serpentinizing subsurface (e.g., Suzuki et al. 2014, Fones et al. 2019). Similarly, CH₄ can derive from acetoclastic and CO₂-, formate- or methanol-based methanogenesis. It also can be produced by anaerobic respiration of organic compounds.

Biological consumption and production of both H₂ and CH₄ are usually tightly coupled through syntrophic relationships, and hydrogenotrophs, hydrogen-producers, as well as methanogens and methanotrophs often coexist and interact in a variety of anoxic habitats (FIG. 3; e.g., Lang et al. 2018; Ranchou-Peyruse et al. 2019). Accordingly, in order to evaluate how and to which extent ecosystems impact natural fluxes of H₂ and CH₄ in environments where abiotic synthesis occurs (Klein et al. 2020 this issue, Reeves and Fiebig 2020 this issue), reliable criteria are needed to assess the origin of H₂ and CH₄.

No dedicated geochemical tools exist to discriminate the biological or abiotic origin of H₂. An abiotic origin is generally attributed to most of the H₂ emissions from rocky environments, although high abundances of fermentative species capable of producing H₂ in some of these environments suggest that this may not always be the case. Further development of omics approaches (i.e., metagenomics, metatranscriptomics and metaproteomics) now allows a quantification of hydrogenase diversity and putative activity in large-scale surveys at reasonable cost (e.g., Lindsay et al. 2019). By reliably documenting the potential for H₂ oxidation or H₂ production, they will allow for a more holistic interpretation of the contribution of deep ecosystems to natural H₂ emissions, of

the associated mechanisms involved, and by extension, of the sustainability of such emissions.

Conversely, significant effort has been applied to developing accurate tools and methodologies to decipher the origin of CH₄ in natural emissions (Reeves and Fiebig 2020 this issue). While the $\delta^{13}\text{C}$ signature of CH₄ has been long considered as a reliable proxy to discriminate between abiotic, biological or thermogenic methane (Etiope and Sherwood Lollar 2013), recently it has been shown that carbon isotopic signatures of abiotic CH₄ associated with serpentinization can be highly similar to those produced by microbial methanogenesis under alkaline conditions that force low levels of dissolved inorganic carbon (Miller et al. 2016). Technological advances that now allow for the sensitive measurement of mass-18 isotopologues of CH₄ hold promise for identifying the processes involved in the production and consumption of CH₄ (e.g., Young 2019). When combined with omics approaches, new insights into biological consumption and production of CH₄ will be obtained that will better inform estimates of natural CH₄ emissions and their origins.

ON THE POTENTIAL ROLE OF H₂- AND CH₄-BASED ECOSYSTEMS IN SUBSURFACE STORAGE

The knowledge accumulated over the past decade on the microbial utilization of H₂ and CH₄, along with the recognition that microbes are widespread in the subsurface (e.g., Magnabosco et al. 2018) and able to thrive under extreme conditions (e.g., Fones et al. 2018) raise questions regarding underground storage of these gases. It has recently been shown that the composition of microbial communities hosted in deep continental aquifers can be irrevocably impacted by decades of transient storage of CH₄-containing domestic gas (Ranchou-Peyruse et al. 2019). Carbon dioxide injections related to carbon capture and storage (CCS) technologies also have been shown to promote fast and pronounced changes in deep basalt-hosted microbial communities and associated metabolisms (Trias et al. 2017). In this Icelandic CCS pilot site, anthropogenic CO₂ injections have been shown to induce microbial blooms of both autotrophs and heterotrophs at depth. Interestingly, they have pointed to the ability of the basalt-hosted ecosystem to potentially grow on PAHs abiotically produced during lava cooling from CO₂, CO and H₂ contained in the magmatic gas and trapped within basalt vesicles and

associated clays. These PAHs may have been released from the host-rock into the groundwater following the acidifying injection of CO₂-charged waters, prompting dissolution of the basalt. Note that the capability of subsurface populations to degrade complex aromatic compounds can be of valuable help for natural and domestic gas storage because the seasonally-stored gas could also contain, in addition to H₂, CO, CO₂, CH₄, and N₂, traces of volatile aromatic compounds of environmental concern that could hence be removed from groundwater by subsurface microbes.

In the near future, H₂ also will be a target for energy storage. Excess power from intermittent energy sources such as solar and wind power can be converted into H₂ through high pressure electrolysis. Underground H₂ storage (UHS) may then provide temporary grid energy storage for an optimal use and improved integration of renewable energy sources. Some of the current geologic targets for UHS are deep cavities in evaporites, including gypsum formations. To avoid impact upon operations, sustainable implementation of such technologies should nonetheless consider the prevalence of hydrogenotrophic sulfate reducers as a major microbial metabolism in the subsurface. In addition to reduced amounts of stored H₂, microbial consumption of H₂ by sulfate reducers could also produce H₂S, an undesired toxic and corrosive compound. Methanogens also could be stimulated by the stored H₂ to convert various carbon sources into CH₄, as is the goal of biotic methanation projects in geological reservoirs. But CH₄ is also a powerful greenhouse gas that could have adverse consequences in the event of a leak into the atmosphere.

IMPLICATIONS FOR EARLIEST METABOLISMS ON EARTH AND ELSEWHERE

The ability of deep microbial ecosystems to thrive on geologically produced H₂ and CH₄ with little or no input from photosynthesis for carbon and energy supply also has far reaching implications for the earliest forms of life on Earth, well before the rise of anoxygenic and oxygenic photosynthetic activity. Subsurface environments are increasingly considered as possible homes to the first forms of terrestrial life, or at least as modern analogs for the environmental settings of early metabolisms. This is because these environments produce highly reduced compounds and hence provide, when in contact with oxidized fluids, disequilibrium in electron donor/acceptor pairs that underpin microbial growth. The subsurface also gives ecosystems the great advantages

of being isolated from a hostile surface exposed to UV irradiation and meteoritic bombardments, as it was the case when life has emerged on Earth.

Primordial metabolisms that can be inferred from modern genomes are commonly thought to have fueled their carbon and energy metabolisms through redox reactions involving H₂ and single-carbon compounds. This is supported by the fact that, while all classes of hydrogenases are well represented within the universal phylogenetic tree of life, only [NiFe]-hydrogenases populate the deepest branches, including the postulated genome of our last universal common ancestor (LUCA, FIG. 5; Peters et al. 2015), suggesting that hydrogenotrophy could represent an ancient characteristic. Hydrogen-based autotrophic methanogenesis and acetogenesis utilizing the acetyl-CoA (or Wood-Ljungdahl) pathway for CO₂ fixation were long considered as the most favorable and ancestral pathways of microbial energy metabolism, the chemical forerunner being represented by geochemically produced methane and/or acetate. However, due to the kinetic inhibition inherent to CH₄ formation at low temperature and the high rate of abiotic CH₄ formation at high temperature (e.g., Andreani and Ménez 2019), it was recently proposed that CH₄ could represent the fuel for, and not the waste of, early life, which possibly implemented a denitrifying methanotrophic acetogenic pathway (Russell and Nitschke 2017). However, it remains unclear what the source of nitrate would have been to fuel such a metabolism. Although the debate is far from being resolved, further investigations of subsurface microbial ecosystems sustained by abiotically-produced sources of carbon and energy may shed light in the near future on the nature of early life on Earth and possibly elsewhere, information that will help guide the search for life on other planetary bodies (Glein and Zolotov 2020 this issue).

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Figure 1 - Metagenomic analytical strategies that allow for the recovery and analysis of taxonomic, functional and phylogenetic information. First metagenomic data are generated from DNA extracted from a microbial community of interest. Various procedures based on marker gene analysis, assembly of 'reads' to 'contigs', and binning of 'contigs' to possible genome reconstructions can then be performed. Finally, metagenome assembled genomes (MAGs) can be subjected to bioinformatics analyses to decipher the metabolic pathways that are encoded in a population, allowing for a prediction of the ecological role of microorganisms, without the need for culturing (modified from Sharpton 2014).

557 Figure 2 - A. Distribution of hydrogenases in microbial genomes, as organized by phyla. B.
558 Distribution of hydrogenases organized by the environment type from where the analyzed
559 metagenomes were recovered. Note that the subsurface is not considered (simplified from
560 Greening et al. 2016).

Figure 3 - Diversity of the metabolic pathways that function in the cycling of H_2 and CH_4 in (deep) microbial ecosystems. This diagram highlights that H_2 and CH_4 (circled in red) can be biologically used and re-generated at many levels (regenerative abiotic processes are not shown). Nox and Sox are oxidized nitrogen (nitrate and nitrite) and sulfur (mainly sulfate and thiosulfate) species whereas $Fe\ ox$ stands for iron oxides. Not represented in the diagram is the possibility to use manganese oxides as an electron acceptor for the oxidation of H_2 and CH_4 . For sake of clarity, rectangles have been colored as in Fig. 4. Dashed lines and italics relate to microbial processes and biologically produced compounds, respectively, whereas plain lines and text in grey indicate compounds produced by geologic processes. OA: organic acids; PAH: polycyclic aromatic hydrocarbons; AA: amino acids; CM: carbonaceous matter. Note that metabolisms based on carbon monoxide (CO) are not indicated although numerous clues for their predominance in serpentinization related environments can be found in the literature (e.g., Fones et al. 2019).

Figure 4 - Catabolic energies available from possible redox reactions in hydrothermal systems hosted in peridotites and basalts (for three different temperatures; adapted from Reveillaud et al. 2016). Logatchev, Lost City, Rainbow, Lucky Strike, TAG belong to the Mid-Atlantic ridge whereas Von Damm and Piccard are located at the Mid-Cayman rise and EPR21 at the East Pacific rise. The data highlight the strong dependence of energetic yields of oxidation-reduction reactions capable of supporting microbial metabolisms on bedrock lithology.

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Figure 5 - Distribution of [FeFe]-hydrogenases (A) and [NiFe]-hydrogenases (B) mapped on a “universal” taxonomic tree of life. In orange are lineages with at least one DNA sequence sharing ancestry in the evolutionary history of life with each hydrogenase isoform (i.e. an homolog). The absence of [FeFe]-hydrogenase homologs in deeply rooted lineages (those that branch near the last universal common ancestor, LUCA) and among Archaea suggests this enzyme was not a property of LUCA. In contrast, the widespread distribution of [NiFe]-hydrogenase homologs among Bacteria and Archaea suggests that this enzyme was present in LUCA genome. Adapted from Peters et al. (2015).