

Abiotic Hydrogen and Methane: Fuels for Life Bénédicte Ménez

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1 FINAL REVISED VERSION

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3 Geologic hydrogen and methane as fuel for life

- 4
- 5 Bénédicte Ménez
- 6 Université de Paris, Institut de physique du globe de Paris, CNRS, Paris, France
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- 8

9 Title Image



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Confocal laser scanning microscopy image of microbial filaments inhabiting juvenile chimneys of
the serpentinization-related hydrothermal field of the Prony bay (New Caledonia). These
filaments (highlighted in green by a fluorescent DNA probe) are mineralized by brucite
(Mg(OH)₂, in blue) and are likely utilizing serpentinization-derived organic compounds (Pisapia
et al. 2017). Courtesy of Emmanuelle Gérard (Université de Paris, Institut de physique du globe
de Paris, France).

18

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.

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

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33 INTRODUCTION

The possibility that reduced compounds such as molecular hydrogen (H₂; Klein et al. 34 2020 this issue) and methane (CH₄; Reeves and Fiebig 2020 this issue) form through 35 geologic processes (e.g., water/rock interaction, water radiolysis, rock fracturing) has 36 strong implications for microbial life throughout Earth history. Molecular hydrogen and 37 38 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 39 microbial communities may exist within geologic formations up to several kilometers 40 deep, as long as temperature (<122°C), water and nutrient requirements are met. Life in 41 these environments might be disconnected from photosynthesis and totally rely on the 42 geosphere for energy and carbon supply. Considering that the vast majority of 43 microorganisms on Earth live in the subsurface (e.g., Magnabosco et al. 2018), abiotic H₂ 44 45 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 46 47 deep biosphere is supported by geologic H₂ and CH₄ remains an open question. Moreover, key questions remain regarding the composition of abiotic organic 48 49 compounds available at depth and their respective utilization by chemosynthetic microbial communities. Low molecular-weight organic acids of abiotic origin, such as 50 formate (HCOO⁻) and acetate (CH₃COO⁻), have been shown to play an important role in 51 supporting communities inhabiting subsurface environments (e.g., Lang et al. 2018, 52 Fones et al. 2019). Recent evidence for abiotic amino acids and carbonaceous matter in 53 54 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).

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Hydrothermal systems driven by water circulation through the oceanic lithosphere are 58 the most intensively studied ecosystems with evidence indicating that their microbial 59 60 inhabitants are sustained by H₂, CH₄, formate, and acetate (e.g., Lang et al. 2018). Such work includes submarine hydrothermal fields along mid-ocean ridges comprising high 61 temperature and acidic basalt-hosted vents, as well as alkaline and warm 62 serpentinization-related vents hosted on mantle-derived rocks (e.g., Adam and Perner 63 2018). Hydrogen- and methane-dependent microorganisms also dominate in 64 groundwater circulating in ophiolites (e.g., Suzuki et al. 2014, Brazelton et al. 2017, 65 Miller et al. 2016;) and in mud volcanoes formed in subduction zones (e.g., Trembath-66 Reichert et al. 2019), two environments where active serpentinization also occurs. 67 Microbial communities relying on a geologic supply of H₂ also have been identified in 68 Precambrian cratons where ancient waters trapped in deep fractures undergo radiolysis 69 during radioactive decay of U, Th, and K in the host rock, producing H₂ and activating the 70 abiotic synthesis of organic compounds (e.g., Li et al. 2016). Chemical reactions between 71 water and freshly broken mineral surfaces can also support episodic or slowly-sustained 72 production of H₂ in a diversity of geological settings undergoing rock fracturing and 73 crushing, including faults or beneath sliding glaciers (e.g., Telling et al. 2015). These H₂ 74 fluxes may support H₂-utilizing communities including methanogens in a large variety of 75 76 rocks, as experimentally demonstrated for granite (Parkes et al. 2018).

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In this article we first review how recent progress in microbial ecology significantly 78 increased our knowledge regarding the microbial utilization of H₂ and CH₄. We 79 80 summarize the reasons why H₂ metabolisms are widespread in a large variety of environments. In almost every system where H₂ and CH₄ are geologically formed, we 81 82 review how these compounds are both biologically consumed and produced, which complicates the assessment of the origin of H₂ and CH₄ as well as the overall influence of 83 H₂- and CH₄-dependent metabolisms on associated global biogeochemical cycles. We 84 then consider the implications for subsurface engineering such as carbon capture and 85 storage or the underground storage of H₂ and CH₄ as energy fuels. Finally, we discuss the 86 87 potential role of geologic H₂ and CH₄ in the earliest metabolisms on Earth.

89 FROM CULTIVATION-BASED APPROACHES TO METAGENOMIC SURVEYS

The capability of microbes to rely on H_2 or CH_4 as an electron donor (or alternatively, to 90 produce these compounds through their metabolism) can be investigated by isolating 91 species using enrichment cultivation techniques, and by measuring consumption and 92 93 production of metabolites. This method represents the oldest and only approach available before the rise of molecular biology methods that target biological 94 macromolecules such as nucleic acids or proteins. Pure cultures make it possible to 95 characterize the physiology of H₂- or CH₄-metabolizing microorganisms, which in turn 96 can inform on their ecology. Experimental work with pure cultures or enriched 97 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 produced CH₄ (e.g., Etiope and Sherwood-Lollar 2013). Deuterium depletion in fatty 101 102 acids also can be a signature of H₂-metabolizing bacteria (e.g., Campbell et al. 2009). However, the difficulty to reproduce appropriate growth conditions combined with the 103 low growth rates and the low microbial cell density typifying subsurface environments 104 (frequently well below 10⁵ cells/ml in groundwaters related to serpentinizing systems – 105 e.g., Brazelton et al. 2017, Fones et al. 2019 – and as low as 10³ cells/gram or less in 106 107 oceanic rocks - Quéméneur et al. 2019), has led to very few environmentally-relevant isolated representatives (e.g., Suzuki et al. 2014, Quéméneur et al. 2019 and references 108 109 therein for serpentinization-related environments). Thus, most microbes from these 110 subsurface and extreme environments remain uncultured.

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

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?").

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As an illustration, microbial H₂-cycling involves the use of hydrogenase proteins, which 125 126 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 127 catalyze the reversible oxidation of H₂ to yield protons and electrons through the 128 reaction $H_2 \leftrightarrow 2H^+ + 2e^-$. They are classified into three main phylogenetically-unrelated 129 classes based on their amino acid sequence, metal content and functionality: the [NiFe]-, 130 131 [FeFe]- and [Fe]-hydrogenases. [NiFe]-hydrogenases can be broken down into several different phylogenetic subclasses that are predictive of their directionality (i.e., H₂ 132 oxidation or/and H₂ production; e.g., Greening et al. 2016, Adam and Perner 2018). 133 Likewise, specific amino acids near the active site of [FeFe]-hydrogenases can, in some 134 cases, be used to infer the ratio between reaction rates for H₂ oxidation and H₂ 135 production. Hence amplification and/or sequencing of hydrogenase encoding genes 136 137 from environmental DNA, when placed within a phylogenetic and bioinformatics framework, allow for the characterization of the potential for H₂ oxidation or production 138 in a community (e.g., Greening et al. 2016, Lindsay et al. 2019). 139

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Similarly, genes encoding proteins involved in microbial CH₄ production or consumption 141 can be used to gauge the potential for these processes in a natural microbial community. 142 Methanotrophy, or the oxidation of CH₄ for use as a source of carbon and energy, can be 143 detected using the gene encoding the alpha subunit of methane monooxygenases 144 (MMOs). The gene encoding the alpha subunit of the methyl coenzyme M reductase 145 146 (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. 147 148 Importantly, some anaerobic CH₄-oxidizing Archaea also use this enzyme, which can nonetheless be readily differentiated from those involved in CH₄ production using 149 evolutionary analysis. Analysis of marker genes then allows one to infer biological 150 functions and to establish relationships between taxonomy and functions. However, the 151 152 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 thecharacterization of poorly studied deep and extreme environments.
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In the past 15 years, the increase in the throughput of metagenomic sequencing and 156 associated computational approaches, combined with a significant reduction in cost, has 157 158 allowed progress in characterizing the taxonomic and functional diversity of microbial communities. Metagenomics — "meta" meaning "beyond" to refer to mixed populations 159 rather than single organisms, and "genomics" meaning the study of genomes — is based 160 on the shotgun sequencing of the total DNA extracted from environmental communities. 161 Instead of targeting only specific marker genes, it provides broader insight into the 162 163 diversity and functional potential of the populations that comprise a microbial community. As illustrated in FIG. 1 (Sharpton 2014), it is achieved by computationally 164 assembling small DNA fragments (i.e., the sequenced 'reads') into larger sequences (i.e., 165 the 'contigs') that can then be binned into metagenome assembled genomes (MAGs). 166 This is followed by analyses of the taxonomy and function of genes encoded in either the 167 'contigs' or the MAGs using bioinformatics approaches. Several thousands of genomes of 168 169 uncultivated Bacteria and Archaea, many from new phyla lacking isolated representatives in laboratory cultures, have been assembled over the past several years. 170 These genomic data have substantially expanded our understanding of microbial 171 diversity and associated metabolic capabilities, how communities are structured, how 172 inter-organism interactions shape ecosystem function, and how microbial diversity 173 associates with and scales across environmental conditions. As an illustration, such an 174 approach was key to ascribing an important role to H₂ in supporting serpentinite-hosted 175 microbial communities (e.g., Adam and Perner 2018 and reference therein) and to 176 suggesting a role for CH₄ cycling in these populations (e.g., Brazelton et al. 2017). 177 178 Importantly, gene surveys of metagenomes enable exploration of the functional potential of a community but does not guarantee that microorganisms are actively 179 180 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., 181 metatranscriptomics) or proteins (i.e., metaproteomics)(e.g., Lindsay et al. 2019). 182 183

184 H₂ AS A KEY DRIVER FOR LIFE

185 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 186 biological membranes, H₂ is an invaluable electron donor for microbial metabolisms. 187 Splitting of H₂ into electrons and protons also allows the generation of proton gradients 188 in cells and hence the synthesis of adenosine triphosphate (ATP), which provides energy 189 190 to drive many processes in living organisms, including autotrophic CO₂ fixation. Conversely, during fermentation and photobiological pathways, the high diffusivity of H₂ 191 allows efficient dissipation of excess reductants from the cell, hence making H₂ a 192 193 common byproduct in these processes.

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195 Contrary to what has long been believed, H₂-based microbial metabolisms are not restricted to specific groups of organisms inhabiting certain types of environments. 196 197 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 198 environments (e.g., Peters et al. 2015, Greening et al. 2016). Hydrogen oxidation can be 199 coupled with the reduction of numerous electron acceptors including CO₂ or HCO₃⁻ from 200 201 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 202 of fueling the energy metabolism of populations inhabiting both anoxic and oxic 203 ecosystems. The large diversity of biochemically distinct hydrogenases with different 204 affinity for H₂ and different sensitivity to O₂ (Peters et al. 2015) allows this reductant to 205 206 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 207 H₂ oxidizers have been isolated from thermally and chemically distinct hydrothermal 208 vents. Similarly, hyperalkaline springs in ophiolites contain numerous H₂ oxidizers 209 210 including the recently isolated, proposed genus *Serpentinomonas* (e.g., Suzuki et al. 211 2014).

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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

218 Proteobacteria, Firmicutes and Actinobacteria and tens of archaeal species from the Euryarchaeota and Crenarchaeota phyla (FIG. 2A). Interestingly, these phyla are well 219 represented in subsurface and hydrothermal ecosystems relying on geochemical energy 220 sources, suggesting that they may play an important role in H₂ consumption or 221 production in these geologic settings (e.g., Adam and Perner 2018). FIG. 2B shows that 222 223 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 224 hydrogenases were estimated to be nearly one order of magnitude higher compared to 225 surface environments (Colman et al. 2017). The potentials of subterranean 226 environments and hydrothermal systems for sustained fluxes of abiotic H₂ (±organic 227 228 compounds) through deep crustal outgassing, crystallization of basaltic lava, water/rock 229 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. 230 3, abiotic H₂ and organic compounds may therefore underlie deep ecosystems, as 231 supported by the recognition of the large contribution of H₂ metabolisms to total 232 ecosystem processes associated with hydrothermal and subsurface environments (e.g., 233 234 Colman et al. 2017, Adam and Perner 2018).

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236 MICROBIAL UTILIZATION OF ABIOTIC H₂ AND CH₄

FIG. 3 illustrates the diversity of the metabolic pathways potentially cycling H₂ and CH₄ 237 in (deep) microbial ecosystems, showing that abiotic H₂ and CH₄ are both valuable 238 electron donors and carbon sources for chemolithotrophic ecosystems functioning with 239 little or no input from photosynthesis. In addition to aerobes oxidizing H₂, abiotic H₂ can 240 fuel autotrophic acetogens and methanogens as well as chemolithotrophs reducing 241 242 oxidized sulfur species such as sulfate, oxidized nitrogen species such as nitrate or iron 243 (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 244 245 electron acceptors. FIG. 3 also highlights the capability of microbial populations to use other geologically-produced organic compounds such as formate, acetate, methanol 246 (CH₃OH) and polycyclic aromatic hydrocarbon (PAHs) as their electron donors instead 247 of H₂ or CH₄ (e.g., Trias et al. 2017, Lang et al. 2018, Fones et al. 2019). Among the latter, 248 249 formate, whose formation is favored during serpentinization at high H_2 levels, is the only 250 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.

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The potential of all these metabolisms to act as primary producers for an entire 254 microbial community is supported by thermodynamic calculations (e.g., Reveillaud et al. 255 256 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 257 and grow — that derives from a naturally occurring process and to provide predictions 258 on electron donor/acceptor pairs that yield the most energy in a given geologic setting. 259 As an illustration, FIG. 4 shows the catabolic energy available in oceanic vents where 260 261 hydrothermal fluids mix with oxidized seawater. It highlights that, compared to basalts, peridotite in contact with oxidized fluids can provide a more energetically favorable 262 landscape for sustaining chemolithotrophic life, including H₂-driven sulfate reduction 263 and methanogenesis, as well as anaerobic CH₄ oxidation (Reveillaud et al. 2016). The 264 higher energy available in hydrothermal fluids derived from the alteration of peridotites 265 is primarily due to higher abundance of abiotic H₂ and CH₄ typically associated with 266 267 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 268 environment strongly depends on the geologic context and the energy sources provided 269 by the host rock lithologies. This alone emphasizes the driving role of the rocky 270 environment in shaping microbial communities. Nevertheless, the host rock also 271 272 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 273 274 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 275 produced energy and carbon sources (e.g., Fones et al. 2019). The abundances, 276 taxonomy and diversity of microbial cells, their functional potential, and their rates of 277 278 substrate utilization, including abiotic H₂ and CH₄, hence strongly depend on the host rock and the extent of interactions with external fluids. 279 280

281 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

283 microbial communities in more diverse subsurface environments including continental

aquifers. One of the most striking examples of the biological utilization of abiotic H₂ can 284 be found in the reduced fracture fluids sampled in deep subterranean environments 285 such as the South African gold mines. The low-diversity microbial community sampled 286 in these fluids at 2.8 to 4.2 km below the Earth's surface is dominated by Desulforudis 287 audaxviator, a Firmicute able to thrive through hydrogenotrophy coupled to sulfate 288 289 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 290 (Li et al. 2016). Water radiolysis, induced by the radioactive decay of natural 291 radionuclides in the host rock, provides disequilibrium in redox reactions to sustain 292 microbial metabolisms in these systems. Radiolysis drives the abiotic production of H₂, 293 294 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 295 Chivian et al. (2008) show that *D. audaxviator* is able to thrive almost alone (constituting 296 297 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 298 mine. H₂-utilizing Betaproteobacteria such as Burkholderiales are also frequently 299 300 associated with methanogens in various geologic settings worldwide. Such an association points to potential competition for abiotic H₂ between autotrophic 301 methanogens and sulfate reducers or alternatively, establishment of symbiotic 302 relationships (e.g., Lang et al. 2018; Ranchou-Peyruse et al. 2019). 303

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305 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.

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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). 317 The recent finding of these potential substrates in serpentinizing environments suggests 318 they may be feedstocks for multiple populations, with the potential to impact natural H₂ 319 and CH₄ budgets. Hydrogen production was demonstrated for fermentative Firmicutes 320 isolated from active alkaline vents in ophiolites and the Firmicute Petrocella 321 322 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 323 CO₂ (Quéméneur et al. 2019 and references therein). Biologically-produced H₂ also can 324 derive from aerobic and anaerobic nitrogen fixation and anaerobic carbon monoxide 325 (CO) oxidation, which are common metabolisms in the serpentinizing subsurface (e.g., 326 327 Suzuki et al. 2014, Fones et al. 2019). Similarly, CH₄ can derive from acetoclastic and 328 CO₂-, formate- or methanol-based methanogenesis. It also can be produced by anaerobic respiration of organic compounds. 329

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Biological consumption and production of both H₂ and CH₄ are usually tightly coupled 331 through syntrophic relationships, and hydrogenotrophs, hydrogen-producers, as well as 332 333 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 334 order to evaluate how and to which extent ecosystems impact natural fluxes of H₂ and 335 CH₄ in environments where abiotic synthesis occurs (Klein et al. 2020 this issue, Reeves 336 and Fiebig 2020 this issue), reliable criteria are needed to assess the origin of H₂ and 337 CH₄. 338

339

No dedicated geochemical tools exist to discriminate the biological or abiotic origin of 340 341 H₂. An abiotic origin is generally attributed to most of the H₂ emissions from rocky 342 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 343 344 development of omics approaches (i.e., metagenomics, metatranscriptomics and metaproteomics) now allows a quantification of hydrogenase diversity and putative 345 activity in large-scale surveys at reasonable cost (e.g., Lindsay et al. 2019). By reliably 346 documenting the potential for H₂ oxidation or H₂ production, they will allow for a more 347 348 holistic interpretation of the contribution of deep ecosystems to natural H₂ emissions, of the associated mechanisms involved, and by extension, of the sustainability of suchemissions.

351

Conversely, significant effort has been applied to developing accurate tools and 352 353 methodologies to decipher the origin of CH₄ in natural emissions (Reeves and Fiebig 2020 this issue). While the δ^{13} C signature of CH₄ has been long considered as a reliable 354 proxy to discriminate between abiotic, biological or thermogenic methane (Etiope and 355 Sherwood Lollar 2013), recently it has been shown that carbon isotopic signatures of 356 357 abiotic CH₄ associated with serpentinization can be highly similar to those produced by microbial methanogenesis under alkaline conditions that force low levels of dissolved 358 inorganic carbon (Miller et al. 2016). Technological advances that now allow for the 359 360 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 361 combined with omics approaches, new insights into biological consumption and 362 production of CH₄ will be obtained that will better inform estimates of natural CH₄ 363 emissions and their origins. 364

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366 ON THE POTENTIAL ROLE OF H₂- AND CH₄-BASED ECOSYSTEMS IN SUBSURFACE 367 STORAGE

The knowledge accumulated over the past decade on the microbial utilization of H₂ and 368 CH₄, along with the recognition that microbes are widespread in the subsurface (e.g., 369 370 Magnabosco et al. 2018) and able to thrive under extreme conditions (e.g., Fones et al. 371 2018) raise questions regarding underground storage of these gases. It has recently been shown that the composition of microbial communities hosted in deep continental 372 aquifers can be irrevocably impacted by decades of transient storage of CH₄-containing 373 domestic gas (Ranchou-Peyruse et al. 2019). Carbon dioxide injections related to carbon 374 capture and storage (CCS) technologies also have been shown to promote fast and 375 pronounced changes in deep basalt-hosted microbial communities and associated 376 metabolisms (Trias et al. 2017). In this Icelandic CCS pilot site, anthropogenic CO₂ 377 injections have been shown to induce microbial blooms of both autotrophs and 378 heterotrophs at depth. Interestingly, they have pointed to the ability of the basalt-hosted 379 ecosystem to potentially grow on PAHs abiotically produced during lava cooling from 380 CO₂, CO and H₂ contained in the magmatic gas and trapped within basalt vesicles and 381

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.

389

In the near future, H₂ also will be a target for energy storage. Excess power from 390 intermittent energy sources such as solar and wind power can be converted into H₂ 391 392 through high pressure electrolysis. Underground H₂ storage (UHS) may then provide temporary grid energy storage for an optimal use and improved integration of 393 394 renewable energy sources. Some of the current geologic targets for UHS are deep cavities in evaporites, including gypsum formations. To avoid impact upon operations, 395 sustainable implementation of such technologies should nonetheless consider the 396 prevalence of hydrogenotrophic sulfate reducers as a major microbial metabolism in the 397 398 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. 399 Methanogens also could be stimulated by the stored H₂ to convert various carbon 400 sources into CH₄, as is the goal of biotic methanation projects in geological reservoirs. 401 But CH₄ is also a powerful greenhouse gas that could have adverse consequences in the 402 403 event of a leak into the atmosphere.

404

405 IMPLICATIONS FOR EARLIEST METABOLISMS ON EARTH AND ELSEWHERE

The ability of deep microbial ecosystems to thrive on geologically produced H₂ and CH₄ 406 407 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 408 409 anoxygenic and oxygenic photosynthetic activity. Subsurface environments are increasingly considered as possible homes to the first forms of terrestrial life, or at least 410 as modern analogs for the environmental settings of early metabolisms. This is because 411 these environments produce highly reduced compounds and hence provide, when in 412 contact with oxidized fluids, disequilibrium in electron donor/acceptor pairs that 413 414 underpin microbial growth. The subsurface also gives ecosystems the great advantages

- 415 of being isolated from a hostile surface exposed to UV irradiation and meteoritic
- 416 bombardments, as it was the case when life has emerged on Earth.
- 417

Primordial metabolisms that can be inferred from modern genomes are commonly 418 thought to have fueled their carbon and energy metabolisms through redox reactions 419 420 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 421 life, only [NiFe]-hydrogenases populate the deepest branches, including the postulated 422 genome of our last universal common ancestor (LUCA, FIG. 5; Peters et al. 2015), 423 suggesting that hydrogenotrophy could represent an ancient characteristic. Hydrogen-424 425 based autotrophic methanogenesis and acetogenesis utilizing the acetyl-CoA (or Wood-Ljungdahl) pathway for CO₂ fixation were long considered as the most favorable and 426 ancestral pathways of microbial energy metabolism, the chemical forerunner being 427 represented by geochemically produced methane and/or acetate. However, due to the 428 kinetic inhibition inherent to CH₄ formation at low temperature and the high rate of 429 abiotic CH₄ formation at high temperature (e.g., Andreani and Ménez 2019), it was 430 431 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 432 and Nitschke 2017). However, it remains unclear what the source of nitrate would have 433 been to fuel such a metabolism. Although the debate is far from being resolved, further 434 investigations of subsurface microbial ecosystems sustained by abiotically-produced 435 sources of carbon and energy may shed light in the near future on the nature of early life 436 on Earth and possibly elsewhere, information that will help guide the search for life on 437 other planetary bodies (Glein and Zolotov 2020 this issue). 438

439

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547 Figure 1 - Metagenomic analytical strategies that allow for the recovery and analysis of 548 taxonomic, functional and phylogenetic information. First metagenomic data are generated 549 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 550 genome reconstructions can then be performed. Finally, metagenome assembled genomes 551 552 (MAGs) can be subjected to bioinformatics analyses to decipher the metabolic pathways 553 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). 554 555



- 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).





562 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 H₂ and CH₄ (circled in red) can 563 564 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 565 sulfate and thiosulfate) species whereas Fe ox stands for iron oxides. Not represented in 566 the diagram is the possibility to use manganese oxides as an electron acceptor for the 567 oxidation of H₂ and CH₄. For sake of clarity, rectangles have been colored as in Fig. 4. 568 Dashed lines and italics relate to microbial processes and biologically produced 569 compounds, respectively, whereas plain lines and text in grey indicate compounds 570 produced by geologic processes. OA: organic acids; PAH: polycyclic aromatic hydrocarbons; 571 572 AA: amino acids; CM: carbonaceous matter. Note that metabolisms based on carbon 573 monoxide (CO) are not indicated although numerous clues for their predominance in 574 serpentinization related environments can be found in the literature (e.g., Fones et al. 2019). 575 576



578

579 Figure 4 - Catabolic energies available from possible redox reactions in hydrothermal systems 580 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-581 Atlantic ridge whereas Von Damm and Piccard are located at the Mid-Cayman rise and 582 EPR21 at the East Pacific rise. The data highlight the strong dependence of energetic yields 583 584 of oxidation-reduction reactions capable of supporting microbial metabolisms on bedrock 585 lithology. 586



589	Figure 5 - Distribution of [FeFe]-hydrogenases (A) and [NiFe]-hydrogenases (B) mapped on a
590	"universal" taxonomic tree of life. In orange are lineages with at least one DNA sequence
591	sharing ancestry in the evolutionary history of life with each hydrogenase isoform (i.e. an
592	homolog). The absence of [FeFe]-hydrogenase homologs in deeply rooted lineages (those
593	that branch near the last universal common ancestor, LUCA) and among Archaea suggests
594	this enzyme was not a property of LUCA. In contrast, the widespread distribution of [NiFe]-
595	hydrogenase homologs among Bacteria and Archaea suggests that this enzyme was present
596	in LUCA genome. Adapted from Peters et al. (2015).
597	