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MINIREVIEW

Molecular Hydrogen, a Neglected Key Driver of Soil Biogeochemical Processes

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ABSTRACT The atmosphere of the early Earth is hypothesized to have been rich in reducing gases such as hydrogen (H_2). H_2 has been proposed as the first electron donor leading to ATP synthesis due to its ubiquity throughout the biosphere as well as its ability to easily diffuse through microbial cells and its low activation energy requirement. Even today, hydrogenase enzymes enabling the production and oxidation of H_2 are found in thousands of genomes spanning the three domains of life across aquatic, terrestrial, and even host-associated ecosystems. Even though H_2 has already been proposed as a universal growth and maintenance energy source, its potential contribution as a driver of biogeochemical cycles has received little attention. Here, we bridge this knowledge gap by providing an overview of the classification, distribution, and physiological role of hydrogenases. Distribution of these enzymes in various microbial functional groups and recent experimental evidence are finally integrated to support the hypothesis that H_2-oxidizing microbes are keystone species driving C cycling along O_2 concentration gradients found in H_2-rich soil ecosystems. In conclusion, we suggest focusing on the metabolic flexibility of H_2-oxidizing microbes by combining community-level and individual-level approaches aiming to decipher the impact of H_2 on C cycling and the C-cycling potential of H_2-oxidizing microbes, via both culture-dependent and culture-independent methods, to give us more insight into the role of H_2 as a driver of biogeochemical processes.

KEYWORDS H_2 oxidation, anaerobic processes, biogeochemical processes, carbon cycle, environmental microbiology, hydrogen, soil

Several prebiotic chemistry models have hypothesized that the primary atmosphere of the early Earth was rich in reducing gases, such as molecular hydrogen (H_2), carbon monoxide (CO), and methane (CH_4) (1–4). The ubiquitous availability of H_2, its low activation energy requirements, and its ability to diffuse through microbial cells led to the hypothesis that H_2 was the first electron donor as a mean to generate an ion gradient and, consequently, ATP synthesis (5, 6). In this regard, the iron-sulfur world hypothesis stipulates that NiFe/FeS active centers similar to the active site of hydrogenase enzymes, catalyzing the interconversion of H_2 into protons and electrons, could have emerged a billion years ago and have led to the first biotic sources and sinks of H_2 (7). The availability of elevated mixing ratios of H_2 in many ecological niches might represent a type of selection pressure maintaining this ancient metabolism within today's microbes, including environmental isolates (e.g., newly cultivated *Verrucomicrobia*) (8) and human pathogens (e.g., *Helicobacter pylori*-associated gastric carcinogenesis) (9). Indeed, hydrogenases are well represented within the tree of life, as they are found in thousands of genomes from over 30 phyla (10, 11) (Fig. 1–3). They support autotrophic and mixotrophic lifestyles in various ecosystems encompassing soil, aquatic, and animal-associated niches (6).

In soils, microbial growth is often limited by the scarcity of carbon sources and nutrients (6), which translates to bursts of growth between intervals of starvation and

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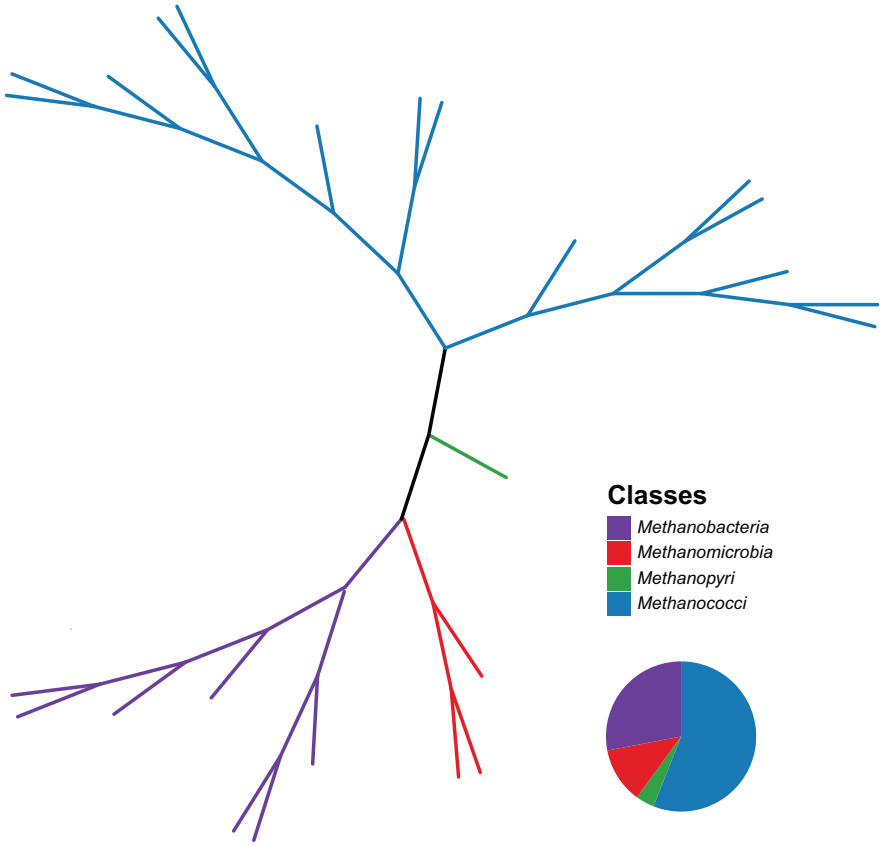

FIG 1 Consensus tree of 25 [Fe]-hydrogenase sequences, with an alignment length of 389 amino acids. Protein sequences were imported from a study by Greening et al. (10), which included nonredundant putative hydrogenase catalytic subunits from cultured and environmental metagenomes sourced from NCBI RefSeq and Joint Genome Institute, Microbial Dark Matter (JGI, MDM) databases. More details are found in the article by Greening et al. (10). See Fig. S1 for complete taxa names and a more detailed phylogenetic tree. Hydrogenase amino acid sequences were aligned with MUSCLE (198) and clustered into phylogenetic trees using the following algorithms: maximum likelihood (Jones-Taylor-Thornton substitution model) using RAxML version 8.2.10 (199), maximum parsimony using PAUP 4.0 (200), and neighbor-joining (Jones-Taylor-Thornton substitution model) using BIONJ (201). CIPRES Science Gateway version 3.3 (202) servers were used for phylogenetic trees construction. Tree branches supported by over 50% of the 1,000 bootstrap replications were represented in the final consensus tree. The consensus tree was built using the "ape" package (203) in R (204). Branch colors represent taxonomic classification at the class level, since all 25 sequences are part of the *Euryarchaeota* phylum, while pie charts show the relative abundance of each class within *Euryarchaeota*.

survival. On the other hand, H_2 is ubiquitous, requires a low activation energy, and can easily permeate microbial cells (6). The ubiquity of H_2 is notably due to its high penetrating power through materials and its continuous abiogenic production in Earth's upper mantle and biogenic production by fermentation, N_2 fixation, and photoproduction (6). H_2 oxidation can be combined with the reduction of a wide variety of oxidants, and therefore, it can be performed in most ecosystems. This combination provides a quick energy fix, via H_2, to overcome those short-to-long intervals of starvation-survival. Several studies have calculated this minimal energy requirement from thermodynamic and bioenergetic perspectives (12–14).

When environmental conditions are not suitable for growth, H_2 oxidation could provide energy to undergo anabiosis, i.e., long-term-stationary phase or even dormancy (6, 15). Such a lifestyle might combine starvation-survival with another survival mechanism, such as anhydrobiosis (i.e., desiccation) or cryobiosis (i.e., freezing) (16), not unlike microbes trapped in amber or frozen in permafrost. Metabolically inactive

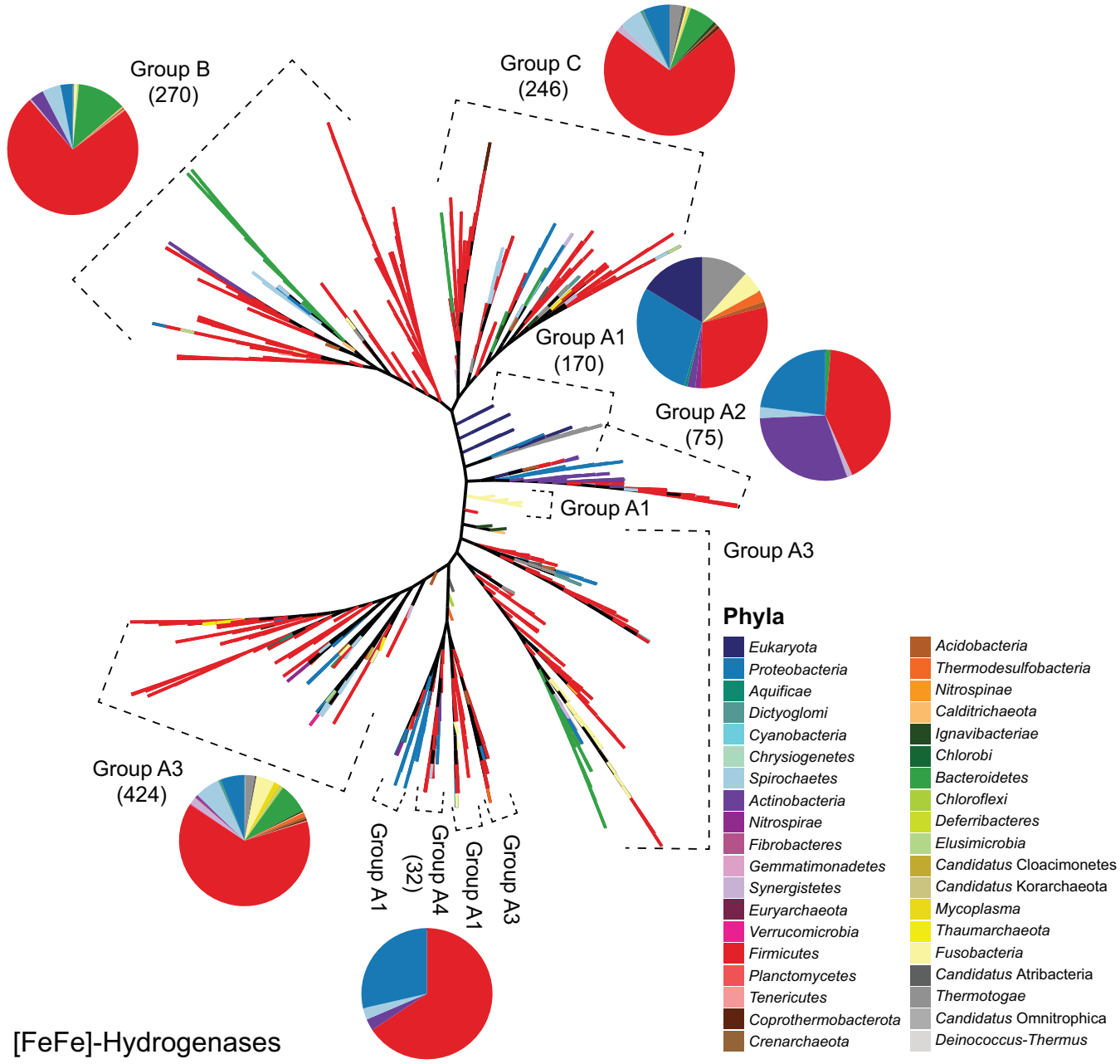

FIG 2 Consensus tree of 1,217 [FeFe]-hydrogenase sequences, with an alignment length of 3,525 amino acids, computed as described in Fig. 1. See Fig. S2 for complete names of taxa and a more detailed phylogenetic tree. Branch colors represent taxonomic classification at the phylum level, while pie charts show the relative abundance of each phylum within hydrogenase subgroups (except for Eukaryota being a superkingdom). Dashed brackets show which tree branches belong to a specific hydrogenases group. Numbers between parentheses depict the amount of sequences within that group.

lifestyles drastically reduce energy demands, yet microbial cells still need to prevent amino acid racemization (i.e., spontaneous change of isomeric form) and DNA depurination over extended periods of time by constantly repairing cellular damage or resynthesizing DNA (13, 14, 17, 18), requiring a continuous input of energy. When organic matter and H_2 abound, H_2-oxidizing microbes (HOM) can switch from short- or long-term dormancy to a more metabolically active lifestyle (6).

While recent studies have shown the ubiquity and importance of H_2 in natural and engineered ecosystems, this field of study has been fairly overlooked since few research groups have focused on biotic H_2 dynamics at the ecosystem level (19). The aim of this review is to examine and discuss the potential role of H_2 as an energy source

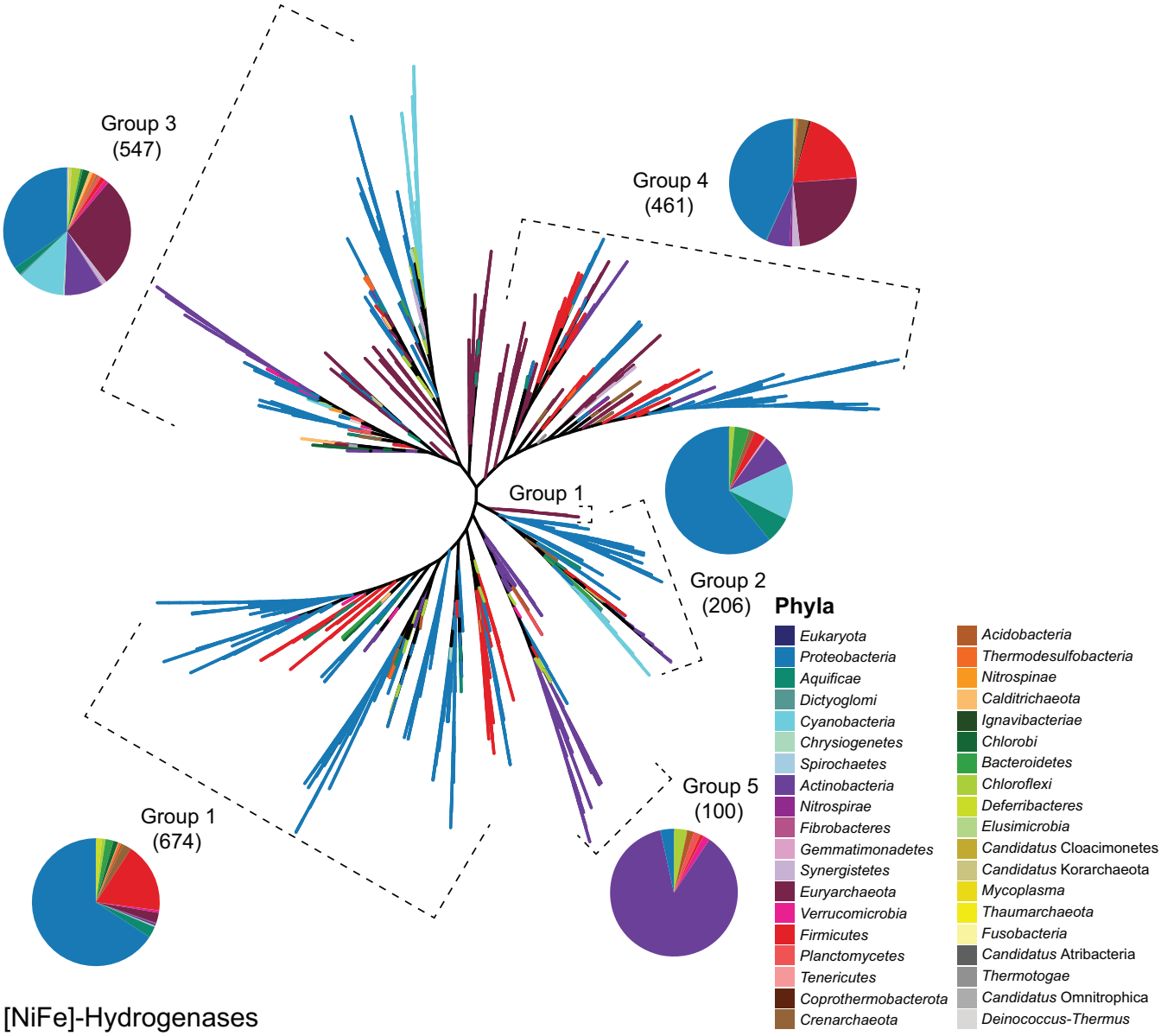

FIG 3 Consensus tree of 1,988 [NiFe]-hydrogenases sequences, with an alignment length of 1,850 amino acids, computed as described in Fig. 1. See Fig. S3 for complete names of taxa and a more detailed phylogenetic tree. Branch colors represent taxonomic classification at the phylum level, while pie charts show the relative abundance of each phylum within hydrogenase subgroups (except for Eukaryota being a superkingdom). Dashed brackets show which tree branches belong to a specific hydrogenases group. Numbers between parentheses depict the amount of sequences within that group.

maintaining microbial viability and activity and, in turn, sustaining soil biogeochemical processes. First, a brief overview of hydrogenase enzymes is provided, including their classification and physiological roles, with emphasis on their kinetic properties and O_2 tolerance. The following section describes functional groups producing and oxidizing H_2 across oxic and anoxic soil habitats. The last section integrates the biochemical properties of hydrogenases to propose succession patterns of HOM along theoretical gradients of O_2 and H_2 prevailing in soils, as well as their potential implications in the carbon cycle. This review concludes with research perspectives aiming to evaluate the contribution of HOM in soil biogeochemical processes.

HYDROGENASE CLASSIFICATION: AN OVERVIEW

Hydrogenases are metalloenzymes catalyzing the interconversion of H_2 to protons and electrons according to the following reaction: $H_2 \rightleftharpoons 2H^+ + 2e^-$ (20). These

enzymes are used either to provide energy, to reduce cofactors, or to disperse reducing equivalents generated through anaerobic processes (11). The conformation of electron bridges between structural subunits and the localization of the protein, membrane-bound or soluble, determine their physiological role (20). Their classification into [Fe]-hydrogenases, [FeFe]-hydrogenases, and [NiFe]-hydrogenases is based on differences between their active sites and amino acid sequences (21). These classes probably originated from converging rather than sequential evolution, since their tertiary structure, catalytic activity, and taxonomic distribution are clearly distinct (22, 23). Horizontal gene transfer is also common in hydrogenases due to their modular structure (24–27). Consequently, several microbes, such as *Cupriavidus necator* (phylum *Proteobacteria*) and *Mycobacterium smegmatis* (phylum *Actinobacteria*), have the genomic capability to express multiple hydrogenases with distinct physiological roles, resulting in an increased metabolic flexibility (28, 29).

(i) [Fe]-hydrogenases. [Fe]-hydrogenases are homodimers consisting of two 38-kDa subunits with a single Fe atom in their catalytic center (20). In contrast with [FeFe]- and [NiFe]-hydrogenases, [Fe]-hydrogenases do not contain Fe-S clusters, are restricted to hydrogenotrophic methanogenic archaea within the *Euryarchaeota* phylum (30) (Fig. 1; see also Fig. S1 in the supplemental material), and are not inactivated by O_2 (31, 32). Under nickel-limiting conditions, [Fe]-hydrogenases work along with F_{420}-dependent methylene tetrahydromethanopterin (methylene-H_4MPT) dehydrogenases to redirect electrons originating from H_2 oxidation toward H_2- and CO_2-dependent methanogenesis (33).

(ii) [FeFe]-hydrogenases. [FeFe]-hydrogenases are mono-, di-, tri-, or tetrameric enzymes containing an active center with two Fe atoms, called H-cluster, as well as Fe-S clusters (34). They are strictly anaerobic since their active site is denatured by O_2 exposure (20). [FeFe]-hydrogenases almost exclusively produce H_2 (28, 35), with a few exceptions, such as in *Desulfovibrio vulgaris* (phylum *Proteobacteria*), whose [FeFe]-hydrogenase can oxidize H_2 under nickel-limited conditions to circumvent [NiFe]-hydrogenase synthesis (36). [FeFe]-hydrogenases are mostly found in anaerobic *Firmicutes* and sulfate reducers (Fig. 2 and S2) due to their O_2 sensitivity (23, 37, 38). They consist of the only class of hydrogenases found in eukaryotes, namely, in the unicellular green alga *Chlamydomonas reinhardtii* (phylum *Chlorophyta*) (39), where they are located within membrane-limited organelles like chloroplasts and hydrogenosomes (40).

(iii) [NiFe]-hydrogenases. [NiFe]-hydrogenases are the most widespread hydrogenase class (11). Several structural and accessory genes are necessary for their maturation (41). While [NiFe]-hydrogenases can be multimeric, they contain at the very least a core heterodimeric component comprising a large α-subunit (ca. 60 kDa) and a small β-subunit (ca. 30 kDa) (20). [NiFe]-hydrogenases represent a heterogenous group of hydrogenases divided into 5 subgroups according to their phylogeny (Fig. 3 and S3) and physiology (20). Among these physiological traits, O_2 tolerance and low catalytic affinity are the most common (20). Indeed, only a few subgroups are sensitive to O_2, and a single subgroup has a high-affinity toward H_2, i.e., it can oxidize atmospheric H_2 (0.53 ppmv [42]). This classification scheme supported by concurrence between gene sequences and physiological roles of hydrogenases has been revisited recently using structural gene sequences similarity networks, suggesting a higher number of putative subclasses within each group (10, 43). Such reclassifications will not be covered here owing to the lack of experimental observations supporting their coherence with genetic, biochemical, and physiological features of hydrogenases.

(a) Group 1 [NiFe]-hydrogenases. Group 1 [NiFe]-hydrogenases (membrane-bound hydrogenases [MBH]) are H_2 uptake hydrogenases attached to the cytoplasmic membrane (periplasmic side) (20). They are intimately linked to the electron transport chain by supplying the quinone pool with electrons via cytochrome *b*, leading to chemiosmosis and the subsequent generation of ATP (44). MBH are used for energy generation by coupling the oxidation of H_2 with the reduction of several electron acceptors, such

as CO_2, SO_4^{2-}, NO_3^-, Fe(III) oxides, or O_2 (44). They are mostly found in *Proteobacteria* and *Firmicutes* (62.5% and 17.3%, respectively) (Fig. 3 and S3). Ancestral MBH found in methanogenic archaea and sulfate-reducing bacteria are O_2 sensitive (44). In contrast, those associated with the Isp complex, in obligate and facultative autotrophs, and with HybA-MBH, in *Gammaproteobacteria*, have a low tolerance toward O_2 (44). O_2-tolerant MBH are used to perform the Knallgas reaction, i.e., oxidation of H_2 using O_2 as terminal electron acceptor, or other reactions involving high-potential electron acceptors such as nitrogen oxides or bacteriochlorophylls (44).

(b) Group 2 [NiFe]-hydrogenases. Group 2 [NiFe]-hydrogenases consist of cytoplasmic enzymes divided into 2 distinct functional groups, 2a and 2b (20). [NiFe]-hydrogenases encompassing group 2a are soluble uptake hydrogenases oxidizing H_2 endogenously produced by N_2 fixation (45) or NADH/NADPH oxidation (29) and are mainly found in *Cyanobacteria* and other N_2-fixing bacteria (45, 46) (Fig. 3 and S3). Their role is to channel electrons to the electron transport chain and supply energy to the cell (34). They are often located inside cyanobacterial heterocysts devoid of oxygenic photosynthesis and are upregulated under anoxic conditions due to their O_2 sensitivity (47). In contrast, group 2b [NiFe]-hydrogenases are H_2-sensing regulatory hydrogenases (RH) acting as two-component signal transduction systems (20). The specific shape and size of the hydrophobic gas channel leading exogenously produced H_2 to the active site confer O_2 tolerance to the enzyme (48). When H_2 is present, RH induce the transcription of group 1 and group 3 uptake hydrogenase operons in Knallgas bacteria (49, 50).

(c) Group 3 [NiFe]-hydrogenases. Group 3 [NiFe]-hydrogenases are cytoplasmic enzymes called bidirectional heteromultimeric [NiFe]-hydrogenases or soluble hydrogenases (SH) (20). Their role is to provide redox balance within the cell in both bacteria and archaea (11). The structural subunits of those enzymes are in close association with protein modules binding soluble cofactors (20). Group 3 [NiFe]-hydrogenases are O_2 tolerant due to two extra cyanide ligands bound to the Ni and Fe atoms of their active site (51). These hydrogenases, mainly found in *Proteobacteria* (37.4%) and *Euryarchaeota* (27.4%) (Fig. 3 and S3), are further divided into 4 subgroups (20). Group 3a [NiFe]-hydrogenases are heterotrimeric enzymes initially found in methanogenic archaea (52), but more recently in some bacterial phyla (53), and are used to reduce the F_{420} cofactor. Group 3b [NiFe]-hydrogenases are tetrameric enzymes acting like bifunctional sulfhydrogenases (20). They were initially found in hyperthermophilic archaea yet have also been detected in bacterial phyla more recently (10). They reduce S^0 to H_2S or oxidize NAD(P)H and produce H_2 to disperse reducing equivalents generated through fermentation, or, alternatively, to produce NADPH by using H_2 (54). Group 3c [NiFe]-hydrogenases are heterodisulfide reductase-associated hydrogenases that catalyze the reduction of compounds such as methyl viologen and coenzyme M-disulfide bond-coenzyme B (CoM-S-S-CoB) (55) and are mostly found in methanogens and sulfate reducers. Group 3d [NiFe]-hydrogenases consist of a heteromeric hydrogenase and a NADH-dehydrogenase module that binds to NAD(P)H (20). The amount of hydrogenase subunits varies greatly, consisting of, for example, a heterodimeric enzyme in *C. necator* (56) or a heteropentameric enzyme in *Cyanobacteria* (57). These hydrogenases are used to balance the NAD^+/NADH pool by reducing NAD^+ using H_2 (20).

(d) Group 4 [NiFe]-hydrogenases. Group 4 [NiFe]-hydrogenases or membrane-associated energy-converting hydrogenase are oxygen-sensitive (58) multimeric enzymes comprising two proteins embedded within the cytoplasmic membrane as well as hydrophilic subunits (20). Group 4 [NiFe]-hydrogenases are mostly found in methanogenic archaea, chemolithoautotrophic bacteria, and hyperthermophilic archaea (Fig. 3 and S3). In facultative chemolithoautotrophs, group 4 hydrogenases disperse reducing equivalents generated through fermentation by coupling the reduction of protons from water with the anaerobic oxidation of C_1 compounds like formate or carbon monoxide (59). In acetoclastic methanogens, energy-converting hydrogenases (Ech) perform the H_2-dependent reduction of ferredoxin, leading to the generation of a proton gradient through the cytoplasmic membrane and the generation of ATP (60). In

methanogens without a cytochrome, group 4 [NiFe]-hydrogenases (Eha and Ehb) reduce ferredoxin and catalyze H_2 oxidation, which is then coupled with the generation of a sodium ion motive force (20). Group 4 [NiFe]-hydrogenases also include energy-converting Mbh-type hyperthermophilic enzymes producing H_2 in order to reestablish oxidized ferredoxin levels (61).

(e) Group 5 [NiFe]-hydrogenases. Group 5 [NiFe]-hydrogenases are O_2-tolerant (62) uptake hydrogenases harboring a high affinity toward H_2 enabling them to oxidize atmospheric H_2 (63). Their activity was first discovered in spores of *Streptomyces* spp. (phylum *Actinobacteria*) (64). These enzymes are likely associated with the cytosolic side of the membrane through an electron acceptor, but conflicting results have been reported (62, 65). Genomic database mining demonstrated that group 5 [NiFe]-hydrogenases are mostly found in *Actinobacteria* (84.0%, Fig. 3 and S3) (10, 66). The proposed role of these hydrogenases is to provide a growth advantage (67) or a supplementary energy source within a survival-mixotrophic lifestyle (68) to sustain microbial seedbanks in oligotrophic environments (66). However, *C. necator* has a weakly expressed group 5 [NiFe]-hydrogenase that cannot oxidize atmospheric H_2 (62). Indeed, biochemical properties conferring high affinity for H_2 are unknown. While there is a high sequence similarity between the active site of group 5 [NiFe]-hydrogenases, such hydrogenases display a wide spectrum of affinities, e.g., K_m over 1,000 ppmv in *C. necator* in contrast to 11 ppmv in *Streptomyces* sp. strain PCB7 (64). This suggests that either a lateral gene transfer in *C. necator* (69) or the evolution of unknown biochemical attributes in hydrogenases were found in high-affinity HOM. Alternatively, other factors, such as the currently unknown physiological electron acceptor of group 5 [NiFe]-hydrogenases (65, 70), could be responsible for their affinity toward H_2. Their affinity has not been tested extensively *in vitro*; thus, we cannot assume that *C. necator* is an exception to the rule. Also, diverging phylogenetic analyses spawned confusion in hydrogenase terminology, yet group 5 (62, 66, 71) and group 1h (10) refer to the same enzymes.

OVERVIEW OF THE H_2 BIOGEOCHEMICAL CYCLE

Biological sources and sinks of H_2 are common in oxic and anoxic ecosystems. In anoxic ecosystems, such as wetlands, freshwaters under the chemocline, marine sediments, and animal gastrointestinal tracts, H_2 is mainly produced as a reaction intermediate of organic matter degradation by organisms from the three domains of life. The main biological sinks of H_2 in anoxic ecosystems are acetogens, methanogens, sulfate-reducing microbes, iron oxide-reducing microbes, and nitrate-reducing microbes (35). In oxic environments, such as upland soils, freshwaters above the chemocline, and open oceans, H_2 is mainly generated by N_2 fixation and is consumed by Knallgas bacteria and high-affinity H_2-oxidizing bacteria (35). Due to its energetic potential, H_2 is quickly consumed by microbes within the same microenvironment, indicating that H_2 production is likely the limiting step of the H_2 biogeochemical cycle (33). Microbial functional groups responsible for H_2 production and utilization in anoxic and oxic ecosystems will be presented in the following subsections to showcase their vast diversity and their involvement in the C biogeochemical cycle.

(i) H_2 production in anoxic ecosystems. In anoxic ecosystems, photoproduction (or biophotolysis), dark fermentation, and photofermentation are responsible for most of the H_2 production (35).

(a) Photoproduction. Photoproduction of H_2 in aquatic sediments is performed by unicellular algae such as *Chlorella fusca* (phylum *Chlorophyta*), *Tetradesmus obliquus* (formerly *Scenedesmus obliquus*; phylum *Chlorophyta*) (72), and *Chlamydomonas reinhardtii* (73), as well as *Cyanobacteria*. In soils, several purple nonsulfur bacteria perform photoproduction, including *Rhodobacter capsulatus* (phylum *Proteobacteria*) (74) and *Rhodopseudomonas rutila* (phylum *Proteobacteria*) (75). This process occurs under oxygen and CO_2 limitation (76, 77). Light-excited electrons are transferred across photosystems to reduce ferredoxin, and then ferredoxin is reoxidized in order to produce H_2 via an [FeFe]-hydrogenase (78). This process maintains the electron flow

within the electron transport chain to resume oxygenic photosynthesis as soon as better conditions arise (77), such as changing tides or the next photoperiod.

(b) Photofermentation and dark fermentation. Unicellular algae such as *Fragilariopsis* spp. (phylum *Bacillariophyta*) and *Pyramimonas* spp. (phylum *Chlorophyta*) (78), strict anaerobes like *Clostridium acetobutylicum* (phylum *Firmicutes*) (79), and even facultative anaerobes like *Escherichia coli* (phylum *Proteobacteria*) (80) from both aquatic and terrestrial environments can produce H_2 through dark fermentation. Glycolysis, followed by the decarboxylation of pyruvate into acetyl-coenzyme A (acetyl-CoA), reduces ferredoxin/NAD^+ into oxidized ferredoxin/NADH, supplying electrons to a hydrogenase and generating H_2 by their reoxidation (78, 81, 82). Alternatively, the conversion of CoA and pyruvate into formate and acetyl-CoA followed by the cleavage of formate also leads to the generation of H_2 and CO_2 (83). Oxidized ferredoxin and NADH are regenerated by discarding reducing equivalents, and glycolysis can generate ATP anew. Photofermentation is performed by purple nonsulfur bacteria (e.g., *Rhodospirillum rubrum* [phylum *Proteobacteria*] or *Rhodobacter sphaeroides*) that use light energy to convert organic substrates, like lactic acid, into H_2 and CO_2 (83). These processes can be performed sequentially, given that there are dark and light cycles. According to genomic surveys and culture-dependent measurements, in aquatic environments, H_2-evolving fermenters include unicellular algae, *Firmicutes*, *Bacteroidetes*, *Spirochaetes,* and *Proteobacteria* (78, 84–86). In anoxic soils, such as paddy fields, molecular surveys targeting [FeFe]-hydrogenases have shown that *Firmicutes* (mostly *Clostridia*), *Proteobacteria* (mostly *Deltaproteobacteria*), and *Chloroflexi* are the most abundant H_2 producers (87, 88). Similarly, in a moderately acidic fen slurry enrichment, fermenters were identified as *Firmicutes* (*Clostridia* and *Negativicutes*) and *Deltaproteobacteria* (89).

(c) Nitrogen fixation. Various microbes, such as *Clostridium* spp. and *Bacillus* spp. (phylum *Firmicutes*) as well as methanogenic archaea and *Cyanobacteria* can fix N_2 and consequently produce H_2 in anoxic or microoxic environments (90, 91). Since current estimates suggest that most of the biological nitrogen fixation is performed in oxic ecosystems (92), especially in surface waters (93, 94) and in the rhizosphere of leguminous and actinorhizal plants (95), it will be discussed in the next section.

(ii) H_2 production in oxic ecosystems. *Nitrogen fixation*. The main biological process responsible for H_2 production in oxic ecosystems is biological N_2 fixation (BNF). BNF is performed by the nitrogenase complex according to the following reaction (96–98):

$$N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2 \quad (1)$$

BNF requires a tremendous amount of energy, i.e., a minimum of 16 ATP per fixed N_2 molecule (2 ATP per electron transferred [98]); thus, it is strictly regulated and used only in the absence of bioavailable N (99). Since nitrogenases are irreversibly inactivated by O_2 (98, 100), microbes can avoid O_2 via temporal or spatial separation strategies, as follows: separation of N_2 fixation and oxygenic photosynthesis (100), avoidance of O_2, high respiration rates, and the use of O_2 transporters to buffer local O_2 concentrations (98, 101–103). H_2 is an obligate by-product of the BNF reaction, requiring approximately 35% of the electron flux used toward BNF (104) and leading to local accumulations in environments such as the legume rhizosphere (105, 106). BNF and hydrogen oxidation are often linked due to their complementary outcomes, since H_2 oxidation coupled to O_2 reduction recovers part of the ATP used toward N_2 fixation and leads to a reduction in O_2 pressure and H_2 buildups, both of which would prevent nitrogen fixation (46, 107). Furthermore, three BNF strategies are used by microbes, including symbiotic N_2 fixation (99, 108), plant-associated N_2 fixation (109), and free-living N_2 fixation (97, 110). Unlike other nitrogen fixers, most leguminous plants symbionts do not possess the genetic capability to recycle H_2 produced through nitrogen fixation (i.e., absence of Hup activity) (111). Studies have hypothesized that the additional energy input provided by H_2 exerts a fertilization effect promoting growth of the host plant (112, 113).

(iii) H_2 uptake in anoxic ecosystems. In anoxic ecosystems, numerous microbial functional groups use H_2 as an electron donor to reduce terminal electron acceptors according to their availability and underlying thermodynamic constraints (33). This section discusses some of them in increasing redox potential order, from acetogenesis to denitrification, although other electron acceptors can be coupled to H_2 oxidation, such as MnO_2 and CrO_4. Many of those microbes are facultative anaerobes, yet these processes are only performed in anoxic ecosystems since the reduction of O_2 would be favored thermodynamically over alternative electron acceptors. These functional groups are not mutually exclusive, i.e., numerous bacteria and archaea are able to perform H_2 oxidation using more than one electron acceptor.

(a) Acetogens. Acetogenesis is performed by several strict anaerobes from the bacterial and archaeal domains of life (114). Acetogens are found in a wide variety of ecosystems: wetlands (115), the gastrointestinal tract of animals (116, 117), oceans, subsurface sediments (118), hypersaline waters (119), and oxic soils (120). While the latter sounds counterintuitive, several acetogens and methanogens can survive in the presence of O_2 due to different mechanisms (121, 122). Most acetogens, namely, *Clostridium* spp., are comprised within the *Firmicutes* (123), yet some are also found among other phyla, including the archaeal phylum *Bathyarchaeota* (114). The acetogenesis reaction (equation 2) follows the Wood-Ljungdahl pathway to convert C_1 inorganic compounds (CO_2) into C_2 organic compounds (acetate) (124):

$$2CO_2 + 4H_2 \rightleftharpoons CH_3COO^- + H^+ + 2H_2O \quad (2)$$

Acetogens scavenge H_2 generated through the degradation of organic matter. Acetogens face fierce competition from methanogens, methanogens having a lower threshold for H_2 utilization and a higher energy yield (125, 126). Acetogens can overcome methanogens in some ecosystems, such as in the termite gut (127), since the production of acetate consists of an ecological advantage in its animal host. Acetogens are inherently involved in H_2 and C-cycling processes by coupling the utilization of CO_2 and H_2. In turn, this leads to the generation of acetate, a bioavailable C source for the surrounding microbial community. As strict or facultative anaerobes, acetogens can also use dissolved organic matter (DOM, also known as labile) through fermentation and produce their own H_2 and CO_2. Additionally, some of them, e.g., *Clostridium carboxidivorans* and *Clostridium drakei* (128, 129), are also involved in CO oxidation to CO_2, crystalline cellulose and hemicellulose degradation, polymeric organic matter (POM, also known as recalcitrant) turnover (130), and humic acid decomposition (131).

(b) Methanogens. Methanogenesis is only performed by methanogenic archaea, which are common in wetlands, in the intestinal tract of animals, in extreme environments, such as hot springs and hydrothermal vents (132), and even in oxic soils (120). Methanogens consist of the only functional group harboring [Fe]-hydrogenases, which enables them to use H_2 as a reducing agent for the ferredoxin-dependent reduction of CO_2 to CH_4, according to the following simplified reaction (133, 134):

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

Methanogens thrive in ecosystems or layers within those ecosystems where electron acceptors with higher redox potential, such as sulfate and nitrate, are depleted due to the low redox potential of CO_2 (33). They scavenge CO_2 and H_2 derived from the degradation of organic matter, which they can ferment on their own. Hydrogenotrophic methanogens using electron bifurcation are considered obligate autotrophs (135). Methylotrophic methanogens using oxidative phosphorylation consume a wider variety of substrates (136). Recent studies, however, have shown that hydrogenotrophic methanogens such as the marine methanogen *Methanococcus maripaludis* (phylum *Euryarchaeota*) (137) might in fact have a greater flexibility than initially thought by using a combination of CO and formate rather than only CO_2 and H_2. The assumption that methanogens are strict anaerobes has also been challenged with the discovery of "*Candidatus* Methanothrix paradoxum" (phylum *Euryarchaeota*) (nonhydrogenotrophic) (138). The involvement of

methanogens in the C cycle also includes their ability to reduce humic acids, e.g., in *Methanosarcina barkeri* (phylum *Euryarchaeota*) (139), which temporarily decreases their methanogenesis potential. Studies even suggest that anaerobic methanotrophic archaea (ANME) could be methanogens as well (140, 141).

(c) Sulfate-reducing bacteria and archaea. Sulfate-reducing bacteria (SRB), such as *Desulfovibrio* spp. and *Desulfotomaculum* spp. (phylum *Firmicutes*), as well as sulfate-reducing archaea (SRA), such as *Archaeoglobus* spp. (phylum *Euryarchaeota*), are anaerobic microbes coupling the oxidation of H_2 (or organic compounds) to the reduction of sulfate (SO_4^{2-}) into sulfide compounds (142, 143).

While they are found in oxic environments, such as coastal sediments (144), sulfate-reducing microbes are more common in marine and freshwater sediments, deep subsurface environments, and the gastrointestinal tract of animals (143). They are also found in acid mine drainages and alkaline environments, such as soda lakes (143). H_2-utilizing sulfate reducers can outcompete acetogens and methanogens within the same ecosystem due to their higher growth kinetics (143). Species of *Desulfitobacterium* (phylum *Firmicutes*) are also able to perform the reduction of humic acids and polyaromatic compounds (145, 146). Studies have also shown their potential ability to fix CO_2 (147, 148) and oxidize CO (149). Sulfate-reducing microbes are thought to be the main drivers of carbon cycling in marine sediments (143). H_2-oxidizing SRB also act as syntrophs in consortia performing the anaerobic oxidation of CH_4 (150).

(d) Iron oxide reducers. Iron oxide-reducing bacteria and archaea [Fe(III) reducers] are found in soils, acid mine effluents (151), aquatic sediments (152), hot springs (153), and subsurface ecosystems. Since Fe(III) is insoluble in water, microbes need direct contact with Fe(III) to reduce it (154). Microbes such as *Shewanella* spp. (phylum *Proteobacteria*), *Geobacter* spp. (phylum *Proteobacteria*), and *Geothrix* spp. (phylum *Acidobacteria*) can produce chelators to solubilize Fe(III) in water or even produce electrically conductive bacterial nanowires to reach it (154). They use Fe(III) as an electron acceptor for the oxidation of organic matter or H_2 (155–157). Many Fe(III) reducers use other electron acceptors, such as O_2, SO_4^{2-}, NO_3^-, or even CO and CO_2 (139, 155). Those Fe(III) reducers include bacteria, such as *Geobacter* spp. and *Shewanella* spp. (152, 155), but also methanogens (139) and other archaea, like *Pyrobaculum islandicum* (phylum *Crenarchaeota*) (158). Fe(III)-reducing processes are more efficient at scavenging H_2 than are sulfate-reducing processes (159). Several Fe(III) reducers can use humic substances as electron acceptors to anaerobically oxidize organic compounds (e.g., acetate produced by acetogens) as well as H_2 to support their growth (160). Such electron transfers also chelate Fe(III) oxides and make them bioavailable to Fe(III) reducers such as *Geobacter metallireducens*, which also contribute to pollutant degradation (e.g., benzene) (160).

(e) Denitrifying bacteria and archaea. Denitrifying bacteria and archaea couple the reduction of nitrate (NO_3^-) and subsequently reduced nitrogen compounds to the oxidation of H_2 (or organic compounds) (161). Denitrification is performed in aquatic and terrestrial ecosystems mostly by heterotrophic microbes, such as *Paracoccus denitrificans* (phylum *Proteobacteria*), but is also quite common in autotrophic microbes, such as *Thiobacillus denitrificans* (phylum *Proteobacteria*), or within the *Hydrogenophilales* order (phylum *Proteobacteria*) (162–164). It is the most energetically favored anaerobic respiration process and is used by a wide variety of microbes (10, 33). Since it is performed in numerous sequential reduction reactions, typically $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$, many denitrifying microbes do not possess the whole metabolic machinery to perform complete denitrification. Within a single ecosystem, different microbial strains often complement their denitrifying capabilities for more efficient energy retrieval and avoidance of toxic N intermediates (165). *Pseudomonas* spp. (phylum *Proteobacteria*) can perform denitrification as well as POM degradation, including degradation of lignin (166) and aromatic compounds (167). Like SRB, some denitrifying bacteria are also syntrophs within AOM consortia (168).

(iv) H_2 uptake in oxic ecosystems. In aerated soils, the oxidation of elevated H_2 mixing ratios is performed by Knallgas bacteria, whereas high-affinity H_2-oxidizing bacteria (HA-HOB) are able to oxidize much lower H_2 mixing ratios, such as atmospheric H_2 (ca. 0.53 ppmv [42]).

(a) Knallgas bacteria. Knallgas bacteria are aquatic and terrestrial obligate or facultative chemolithoautotrophs that use O_2 as a final electron acceptor to oxidize H_2 according to the Knallgas reaction:

$$H_2 + O_2 \rightleftharpoons H_2O \quad (4)$$

Model Knallgas microbes include *C. necator* (formerly *Ralstonia eutropha*), which possesses 4 [NiFe]-hydrogenases from groups 1, 2, 3, and 5 (28, 62, 169), yet not all Knallgas bacteria have several hydrogenases. While *C. necator* possesses a group 5 [NiFe]-hydrogenase, it cannot oxidize atmospheric H_2 mixing ratios (62) and is thus not a HA-HOB, as opposed to *Mycobacterium smegmatis* (65). When H_2 concentrations are sufficiently high, *C. necator* fixes CO_2 and oxidizes H_2 as its sole energy source using its group 1 [NiFe]-hydrogenase (51). The group 3 [NiFe]-hydrogenase is rather used to maintain redox balance within the cell by coupling H_2 oxidation to NAD^+ reduction (28). To avoid wasting energy by using group 1 and 3 hydrogenases constitutively when H_2 mixing ratios are low, *C. necator* also produces a group 2 hydrogenase acting as a sensor regulating the transcription of structural and auxiliary genes of the two other hydrogenases (28). Finally, the group 5 hydrogenase generates ATP at a lower rate than the MBH (group 1) but is entirely insensitive to O_2 (62). Other Knallgas bacteria have similar H_2 uptake mechanisms and encompass various phylogenetic backgrounds (e.g., *Proteobacteria*, *Firmicutes*, *Cyanobacteria*, *Chloroflexi*, and *Acidobacteria*). Furthermore, many N_2-fixing microbes, namely, the actinorhizal plant symbionts *Frankia* spp. (phylum *Actinobacteria*) and *Cyanobacteria*, can oxidize H_2 produced through N_2 fixation (170). Various Knallgas bacteria are also known plant growth-promoting rhizobacteria (PGPR) (112, 171), while many more perform crucial C-cycling steps. Several Knallgas bacteria can break down polymeric compounds. For instance, *Bacillus pseudofirmus* and *Bacillus thermotolerans* can reduce humic acids when oxidizing Fe(III) oxides (172, 173), while *Burkholderia* spp. (phylum *Proteobacteria*) can break down lignin (166). Furthermore, many methanotrophs, such as *Methylosinus trichosporium* (phylum *Proteobacteria*) and *Methylacidiphilum fumariolicum* (phylum *Verrucomicrobia*), can oxidize H_2 and CH_4 under oxic conditions (174, 175) and are thus also Knallgas bacteria. Various Knallgas bacteria, such as *Hydrogenomonas facilis* (phylum *Proteobacteria*) (176) and *C. necator* (177), can also fix CO_2 or oxidize CO (178).

(b) Atmospheric H_2 oxidation. The oxidation of atmospheric H_2 has long been considered to originate from abiotic enzymes due to the failure to isolate microbes responsible for this process at low H_2 partial pressure (179, 180). Bacteria responsible for the oxidation of H_2 at low mixing ratios have only been recently isolated (64), hence their small representation in the scientific literature. Recent genomic database surveys (10) have shown that most microbes possessing group 5 [NiFe]-hydrogenases are *Actinomycetes,* such as *Streptomyces* spp. and *Mycobacterium* spp. Only a few high-affinity H_2-oxidizing microbes have been isolated and characterized so far, including *Rhodococcus equi* (phylum *Actinobacteria*) (181), various *Streptomyces* spp. (64, 68, 181), and *Mycobacterium smegmatis* (65). In this regard, the role of group 5 [NiFe]-hydrogenases within the cell is not understood. HA-HOB are mostly found in soils, and they represent a major (70%) sink of atmospheric H_2 (182). While atmospheric H_2 provides substantial amounts of energy, it is insufficient for bacterial growth (63). So far, HA-HOB have been shown to use H_2 as an additional energy source for enhanced growth and survival (67, 181) or within a mixotrophic lifestyle called survival mixotrophy (68). HA-HOB are also involved in C cycling. For instance, *Rhodococcus jostii* and *Rhodococcus equi* can degrade lignin and several other POM compounds (166, 183). *Streptomyces* species are also known to perform the breakdown of humic acids and even coal, thus contributing to POM cycling (184–186). Other *Actinobacteria,* such as *Mycobacterium smegmatis*, can also oxidize atmospheric CO and H_2 (187). Candidate

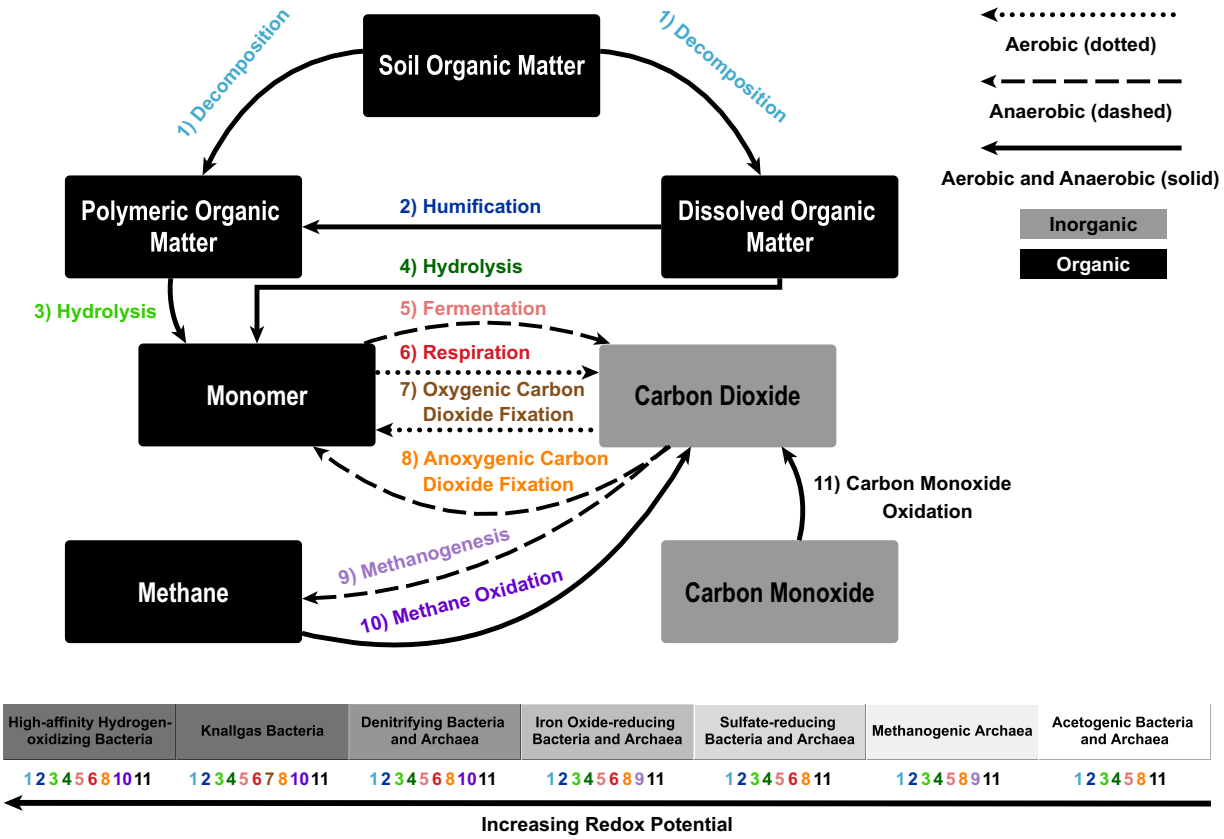

FIG 4 Juxtaposition of the carbon cycle and main H_2-oxidizing functional groups. HOM contribute to all key steps of the C cycle. All numbers below microbial functional groups consist of reactions performed by these groups. While the oxidation of CH_4 can be performed in anoxic ecosystems, the anaerobic oxidation of CH_4 (AOM) is not performed by HOM, only by their syntrophic bacterial partners (i.e., sulfate- or nitrate-reducing bacteria). H_2-utilizing processes occur in a thermodynamically favored fashion according to available substrates, where O_2 is used first, followed by nitrate, iron oxides, sulfate, and carbon dioxide, as shown in the gradient at the bottom of the figure. The only key C-cycling process missing in oxic ecosystems is methanogenesis, yet residual CH_4 diffuses from anoxic to oxic layers in various ecosystems. An exception to this is that nonhydrogenotrophic methanogens are also active in oxic layers (138), thus providing CH_4 directly to oxic ecosystems.

taxa might also have the genetic potential to oxidize atmospheric H_2 and perform CO_2 fixation (188).

JUXTAPOSITION OF THE H_2 AND C BIOGEOCHEMICAL CYCLES IN SOILS ALONG THEORETICAL O_2 GRADIENTS

A wide variety of HOM can hydrolyze DOM but also take part in the breakdown of POM, including cellulose, hemicellulose, lignin, humic substances, and polycyclic aromatic compounds (189), and also catalyze the oxidoreduction of CO, CO_2, and CH_4 (Fig. 4). HOM are able to switch between different modes of nutrition, both in terms of carbon and energy sources (6), which confers them great metabolic versatility when subject to various C inputs. Many of these C-cycling processes also lead to the generation of C sources usable by the broader microbial community, such as acetate. Moreover, in microenvironments rich in H_2, e.g., the legume rhizosphere, termite gut, and serpentinizing systems, metabolites released by HOM might also prime the decomposition and turnover of organic matter, namely, in soils. Recent studies have shown that the exposure of soil to elevated H_2 concentrations representative of those found in nature (i.e., 10,000 ppmv) increased the amount of C sources used, as well as the intensity of their consumption within a defined time period (190, 191). However, it is not known if HOM involved in H_2 oxidation are responsible for this priming-effect-like response due to their mixotrophic growth strategy or if positive interactions between HOM and heterotrophic non-HOM led to it. With this in mind, it could be expected that

other C-cycling processes performed by HOM, such as decomposition, humification, and hydrolysis, could also increase in H_2-rich ecosystems (Fig. 4).

As for trace gases, a study has shown that atmospheric CO and H_2 deposition velocities are positively correlated in both arable and forest soils (192, 193). While the initial hypothesis was that diffusion was ultimately responsible for this response, a more recent study has shown that H_2, CO, and CH_4 oxidation potential by microbes covaries as well. Indeed, along an increasing gradient of H_2 exposure from 0.5 to 10,000 ppmv H_2, low-affinity H_2 oxidation increased, net CO_2 production decreased, and high-affinity H_2, CO, and CH_4 oxidation decreased (194). CO_2 production results were not published but were measured at the same time and with the same instrument as the other trace gases (194). Taken together, those studies imply that ecosystems rich in H_2 could be hubs of DOM and POM cycling; HOM oxidize H_2 preferentially if available at elevated mixing ratios instead of using other trace gases.

While potential activity does not guarantee that *in situ* measurements will show the same behavior, examples of H_2-driven C cycling exist. Two studies have shown that CO_2 dynamics in soil systems continuously fed with air or air supplemented with H_2 change from net CO_2 production to net CO_2 fixation during H_2 exposure (195, 196). A recent metagenomic survey has hypothesized similar outcomes in Antarctic desert soils (188). Fixed CO_2 would increase the input of organic carbon in the system, which can then be distributed to the rest of the microbial community after cell death or production of secondary metabolites. Responsible strains in these studies have not been identified, yet several Knallgas bacteria are known to oxidize H_2 and fix CO_2. While these hypotheses are applied to H_2-oxidizing CO_2-fixing microbes, it is expected that H_2-oxidizing methanotrophs could also favor certain metabolic pathways in the presence of H_2 by decreasing CH_4 oxidation and thus increasing net C input by methanogens (Fig. 4) (194).

While HOM are involved in various C cycling processes, their relative contribution to each of them is unknown. Two different types of approaches could be used to increase our understanding of H_2-C dynamics, community-level and individual-level approaches. As for community-level approaches, a good starting point would be to expose soil (i.e., microcosms, mesocosms, or even fields) to a baseline mixing ratio of H_2 (e.g., 0.53 as control [42]), as well as to lower and higher mixing ratios representative of natural conditions. Soil H_2 exposure could be provided by synthetic gas mixtures or through the manipulation of N_2-fixing H_2-producing rhizobia (with or without Hup activity as isogenic control and treatment strains, respectively) recruited by legume plants. This would enable a direct comparison between H_2 availability and C turnover. As for individual-level approaches, three experiments could be performed, as follows: culturing novel HOM, single-cell sequencing of novel uncultured HOM, or analyzing metagenome-assembled genomes (MAGs) of uncultured HOM. Traditional cultivation would allow for a more direct testing of HOM C-cycling capabilities, while culture-independent methods would give us more insight into their overall versatility. Furthermore, several H_2 and C-cycling processes seem to covary in soil, such as atmospheric CH_4 and elevated H_2 oxidation, yet it is not known if these processes are performed by the same microbes or simply within the same ecological niches. The three proposed individual-level methods could help solve this conundrum, all the while reducing current biases in genomic databases. For example, some taxa have more sequenced representatives and are consequently more represented in databases regardless of their involvement in the cycle (e.g., *Actinomycetales* and high-affinity H_2 oxidation). Further work in these directions would shed light on the importance of the overlap between the C and H_2 cycle at the species and ecosystem level as well as provide further insights into the overall biogeochemical potential of HOM. In turn, this knowledge could be used to develop techniques that take advantage of H_2 hot spots in soils, e.g., for potential increases in C turnover or for agricultural benefits, namely, in legume rhizosphere rich in H_2 (197). Last, this review exemplifies the idea that HOM, as a functional group, is a reductionist concept. That is, HOM should not be reduced to their H_2-oxidizing function; they are highly diverse, and many of them perform crucial ecosys-

tem functions that could be more important at the ecosystem level than their individual energy-retrieving potential.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.02418-18.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

REFERENCES

1. Trainer MG. 2013. Atmospheric prebiotic chemistry and organic hazes. Curr Org Chem 17:1710–1723. https://doi.org/10.2174/13852728113179990078.
2. Oparin AI. 1938. The origin of life. J Chem Educ 15:399. https://doi.org/10.1021/ed015p399.1.
3. Tian F, Toon OB, Pavlov AA, De Sterck H. 2005. A hydrogen-rich early earth atmosphere. Science 308:1014–1017. https://doi.org/10.1126/science.1106983.
4. Zahnle K, Schaefer L, Fegley B. 2010. Earth's earliest atmospheres. Cold Spring Harb Perspect Biol 2:a004895. https://doi.org/10.1101/cshperspect.a004895.
5. Lane N, Allen JF, Martin W. 2010. How did LUCA make a living? Chemiosmosis in the origin of life. Bioessays 32:271–280. https://doi.org/10.1002/bies.200900131.
6. Morita RY. 1999. Is H_2 the universal energy source for long-term survival? Microb Ecol 38:307–320.
7. Wächtershäuser G. 2010. Chemoautotrophic origin of life: the iron–sulfur world hypothesis, p 1–35. *In* Barton LL, Mandl M, Loy A (ed), Geomicrobiology: molecular and environmental perspective. Springer Netherlands, Dordrecht, The Netherlands.
8. Carere CR, Hards K, Houghton KM, Power JF, McDonald B, Collet C, Gapes DJ, Sparling R, Boyd ES, Cook GM, Greening C, Stott MB. 2017. Mixotrophy drives niche expansion of verrucomicrobial methanotrophs. ISME J 11:2599–2610. https://doi.org/10.1038/ismej.2017.112.
9. Wang G, Romero-Gallo J, Benoit SL, Piazuelo MB, Dominguez RL, Morgan DR, Peek RM, Maier RJ. 2016. Hydrogen metabolism in Helicobacter pylori plays a role in gastric carcinogenesis through facilitating CagA translocation. mBio 7:e01022-16. https://doi.org/10.1128/mBio.01022-16.
10. Greening C, Biswas A, Carere CR, Jackson CJ, Taylor MC, Stott MB, Cook GM, Morales SE. 2016. Genomic and metagenomic surveys of hydrogenase distribution indicate H_2 is a widely utilised energy source for microbial growth and survival. ISME J 10:761–777. https://doi.org/10.1038/ismej.2015.153.
11. Vignais PM, Billoud B. 2007. Occurrence, classification, and biological function of hydrogenases: an overview. Chem Rev 107:4206–4272. https://doi.org/10.1021/cr050196r.
12. Hoehler TM, Alperin MJ, Albert DB, Martens CS. 2001. Apparent minimum free energy requirements for methanogenic archaea and sulfate-reducing bacteria in an anoxic marine sediment. FEMS Microbiol Ecol 38:33–41. https://doi.org/10.1111/j.1574-6941.2001.tb00879.x.
13. Alperin MJ, Hoehler TM. 2009. Anaerobic methane oxidation by archaea/sulfate-reducing bacteria aggregates: 2. Isotopic constraints. Am J Sci 309:958–984. https://doi.org/10.2475/10.2009.02.
14. Hoehler TM, Jørgensen BB. 2013. Microbial life under extreme energy limitation. Nat Rev Microbiol 11:83. https://doi.org/10.1038/nrmicro2939.
15. Keilin D. 1959. The problem of anabiosis or latent life: history and current concept. Proc R Soc Lond B Biol Sci 150:149–191.
16. Finkel SE. 2006. Long-term survival during stationary phase: evolution and the GASP phenotype. Nat Rev Microbiol 4:113. https://doi.org/10.1038/nrmicro1340.
17. Poinar HN, Hoss M, Bada JL, Paabo S. 1996. Amino acid racemization and the preservation of ancient DNA. Science 272:864–866.
18. Onstott TC, Phelps TJ, Colwell FS, Ringelberg D, White DC, Boone DR, McKinley JP, Stevens TO, Long PE, Balkwill DL, Griffin WT, Kieft T. 1998. Observations pertaining to the origin and ecology of microorganisms recovered from the deep subsurface of Taylorsville Basin, Virginia. Geomicrobiol J 15:353–385. https://doi.org/10.1080/01490459809378088.
19. Meredith LK, Commane R, Keenan TF, Klosterman ST, Munger JW, Templer PH, Tang JW, Wofsy SC, Prinn RG. 2017. Ecosystem fluxes of hydrogen in a mid-latitude forest driven by soil microorganisms and plants. Glob Change Biol 23:906–919. https://doi.org/10.1111/gcb.13463.
20. Constant P, Hallenbeck PC. 2013. Chapter 5–hydrogenase, p 75–102. *In* Pandey A, Chang J-S, Hallenbeck PC, Larroche C (ed), Biohydrogen. Elsevier, Amsterdam, The Netherlands.
21. Armstrong FA, Albracht SPJ. 2005. [NiFe]-hydrogenases: spectroscopic and electrochemical definition of reactions and intermediates. Philos Trans A Math Phys Eng Sci 363:937–954. https://doi.org/10.1098/rsta.2004.1528.
22. Vignais PM. 2008. Hydrogenases and H^+-reduction in primary energy conservation. Results Probl Cell Differ 45:223–252. https://doi.org/10.1007/400_2006_027.
23. Vignais PM, Billoud B, Meyer J. 2001. Classification and phylogeny of hydrogenases. FEMS Microbiol Rev 25:455–501. https://doi.org/10.1111/j.1574-6976.2001.tb00587.x.
24. Calteau A, Gouy M, Perriere G. 2005. Horizontal transfer of two operons coding for hydrogenases between bacteria and archaea. J Mol Evol 60:557–565. https://doi.org/10.1007/s00239-004-0094-8.
25. Ludwig M, Schulz-Friedrich R, Appel J. 2006. Occurrence of hydrogenases in cyanobacteria and anoxygenic photosynthetic bacteria: implications for the phylogenetic origin of cyanobacterial and algal hydrogenases. J Mol Evol 63:758–768. https://doi.org/10.1007/s00239-006-0001-6.
26. Hansen M, Perner M. 2016. Hydrogenase gene distribution and H_2 consumption ability within the *Thiomicrospira* lineage. Front Microbiol 7:99. https://doi.org/10.3389/fmicb.2016.00099.
27. Keeling PJ. 2009. Functional and ecological impacts of horizontal gene transfer in eukaryotes. Curr Opin Genet Dev 19:613–619. https://doi.org/10.1016/j.gde.2009.10.001.
28. Burgdorf T, Lenz O, Buhrke T, van der Linden E, Jones AK, Albracht SP, Friedrich B. 2005. [NiFe]-hydrogenases of Ralstonia eutropha H16: modular enzymes for oxygen-tolerant biological hydrogen oxidation. J Mol Microbiol Biotechnol 10:181–196. https://doi.org/10.1159/000091564.
29. Berney M, Greening C, Hards K, Collins D, Cook GM. 2014. Three different [NiFe]-hydrogenases confer metabolic flexibility in the obligate aerobe *Mycobacterium smegmatis*. Environ Microbiol 16:318–330. https://doi.org/10.1111/1462-2920.12320.
30. Zirngibl C, Van Dongen W, Schworer B, Von Bunau R, Richter M, Klein A, Thauer RK. 1992. H_2-forming methylenetetrahydromethanopterin dehydrogenase, a novel type of hydrogenase without iron-sulfur clusters in methanogenic archaea. Eur J Biochem 208:511–520.
31. Lyon EJ, Shima S, Buurman G, Chowdhuri S, Batschauer A, Steinbach K, Thauer RK. 2004. UV-A/blue-light inactivation of the 'metal-free' hydrogenase (Hmd) from methanogenic archaea. FEBS J 271:195–204. https://doi.org/10.1046/j.1432-1033.2003.03920.x.
32. Fontecilla-Camps JC, Volbeda A, Cavazza C, Nicolet Y. 2007. Structure/function relationships of [NiFe]- and [FeFe]-hydrogenases. Chem Rev 107:4273–4303. https://doi.org/10.1021/cr050195z.
33. Thauer RK, Klein AR, Hartmann GC. 1996. Reactions with molecular hydrogen in microorganisms: evidence for a purely organic hydrogenation catalyst. Chem Rev 96:3031–3042.
34. Vignais PM, Colbeau A. 2004. Molecular biology of microbial hydrogenases. Curr Issues Mol Biol 6:159–188.
35. Thauer RK. 2011. Hydrogenases and the global H_2 cycle. Eur J Inorg Chem 2011:919–921. https://doi.org/10.1002/ejic.201001255.
36. Pohorelic BK, Voordouw JK, Lojou E, Dolla A, Harder J, Voordouw G. 2002. Effects of deletion of genes encoding Fe-only hydrogenase of *Desulfovibrio vulgaris* Hildenborough on hydrogen and lactate metabolism. J Bacteriol 184:679–686. https://doi.org/10.1128/JB.184.3.679-686.2002.

37. Adams MW. 1990. The structure and mechanism of iron-hydrogenases. Biochim Biophys Acta 1020:115–145.
38. Atta M, Meyer J. 2000. Characterization of the gene encoding the [Fe]-hydrogenase from *Megasphaera elsdenii*. Biochim Biophys Acta 1476:368–371. https://doi.org/10.1016/S0167-4838(99)00245-9.
39. Happe T, Mosler B, Naber JD. 1994. Induction, localization and metal content of hydrogenase in the green alga *Chlamydomonas reinhardtii*. Eur J Biochem 222:769–774.
40. Akhmanova A, Voncken F, van Alen T, van Hoek A, Boxma B, Vogels G, Veenhuis M, Hackstein JH. 1998. A hydrogenosome with a genome. Nature 396:527–528. https://doi.org/10.1038/25023.
41. Lacasse MJ, Zamble DB. 2016. [NiFe]-hydrogenase maturation. Biochemistry 55:1689–1701. https://doi.org/10.1021/acs.biochem.5b01328.
42. Novelli PC, Lang PM, Masarie KA, Hurst DF, Myers R, Elkins JW. 1999. Molecular hydrogen in the troposphere: global distribution and budget. J Geophys Res 104:30427–30444. https://doi.org/10.1029/1999JD900788.
43. Søndergaard D, Pedersen CNS, Greening C. 2016. HydDB: a web tool for hydrogenase classification and analysis. Sci Rep 6:34212. https://doi.org/10.1038/srep34212.
44. Pandelia M-E, Lubitz W, Nitschke W. 2012. Evolution and diversification of group 1 [NiFe] hydrogenases. Is there a phylogenetic marker for O_2-tolerance? Biochim Biophys Acta 1817:1565–1575. https://doi.org/10.1016/j.bbabio.2012.04.012.
45. Tamagnini P, Leitão E, Oliveira P, Ferreira D, Pinto F, Harris DJ, Heidorn T, Lindblad P. 2007. Cyanobacterial hydrogenases: diversity, regulation and applications. FEMS Microbiol Rev 31:692–720. https://doi.org/10.1111/j.1574-6976.2007.00085.x.
46. Bothe H, Schmitz O, Yates MG, Newton WE. 2010. Nitrogen fixation and hydrogen metabolism in *cyanobacteria*. Microbiol Mol Biol Rev 74:529–551. https://doi.org/10.1128/MMBR.00033-10.
47. Axelsson R, Lindblad P. 2002. Transcriptional regulation of *Nostoc* hydrogenases: effects of oxygen, hydrogen, and nickel. Appl Environ Microbiol 68:444–447. https://doi.org/10.1128/AEM.68.1.444-447.2002.
48. Buhrke T, Lenz O, Krauss N, Friedrich B. 2005. Oxygen tolerance of the H_2-sensing [NiFe] hydrogenase from *Ralstonia eutropha* H16 is based on limited access of oxygen to the active site. J Biol Chem 280:23791–23796. https://doi.org/10.1074/jbc.M503260200.
49. Lenz O, Bernhard M, Buhrke T, Schwartz E, Friedrich B. 2002. The hydrogen-sensing apparatus in *Ralstonia eutropha*. J Mol Microbiol Biotechnol 4:255–262.
50. Buhrke T, Lenz O, Porthun A, Friedrich B. 2004. The H_2-sensing complex of *Ralstonia eutropha*: interaction between a regulatory [NiFe] hydrogenase and a histidine protein kinase. Mol Microbiol 51:1677–1689. https://doi.org/10.1111/j.1365-2958.2003.03933.x.
51. Burgdorf T, Loscher S, Liebisch P, Van der Linden E, Galander M, Lendzian F, Meyer-Klaucke W, Albracht SP, Friedrich B, Dau H, Haumann M. 2005. Structural and oxidation-state changes at its nonstandard Ni-Fe site during activation of the NAD-reducing hydrogenase from *Ralstonia eutropha* detected by X-ray absorption, EPR, and FTIR spectroscopy. J Am Chem Soc 127:576–592. https://doi.org/10.1021/ja0461926.
52. Peters JW, Schut GJ, Boyd ES, Mulder DW, Shepard EM, Broderick JB, King PW, Adams MWW. 2015. [FeFe]- and [NiFe]-hydrogenase diversity, mechanism, and maturation. Biochim Biophys Acta 1853:1350–1369. https://doi.org/10.1016/j.bbamcr.2014.11.021.
53. Ney B, Ahmed FH, Carere CR, Biswas A, Warden AC, Morales SE, Pandey G, Watt SJ, Oakeshott JG, Taylor MC, Stott MB, Jackson CJ, Greening C. 2017. The methanogenic redox cofactor F_{420} is widely synthesized by aerobic soil bacteria. ISME J 11:125. https://doi.org/10.1038/ismej.2016.100.
54. Ma K, Schicho RN, Kelly RM, Adams MW. 1993. Hydrogenase of the hyperthermophile *Pyrococcus furiosus* is an elemental sulfur reductase or sulfhydrogenase: evidence for a sulfur-reducing hydrogenase ancestor. Proc Natl Acad Sci U S A 90:5341–5344.
55. Thauer RK, Kaster AK, Goenrich M, Schick M, Hiromoto T, Shima S. 2010. Hydrogenases from methanogenic archaea, nickel, a novel cofactor, and H_2 storage. Annu Rev Biochem 79:507–536. https://doi.org/10.1146/annurev.biochem.030508.152103.
56. Lauterbach L, Liu J, Horch M, Hummel P, Schwarze A, Haumann M, Vincent KA, Lenz O, Zebger I. 2011. The hydrogenase subcomplex of the NAD^+-reducing [NiFe]-hydrogenase from *Ralstonia eutropha*–insights into catalysis and redox interconversions. Eur J Inorg Chem 2011:1067–1079. https://doi.org/10.1002/ejic.201001053.
57. Puggioni V, Tempel S, Latifi A. 2016. Distribution of hydrogenases in cyanobacteria: a phylum-wide genomic survey. Front Genet 7:223. https://doi.org/10.3389/fgene.2016.00223.
58. Bonam D, Lehman L, Roberts GP, Ludden PW. 1989. Regulation of carbon monoxide dehydrogenase and hydrogenase in *Rhodospirillum rubrum*: effects of CO and oxygen on synthesis and activity. J Bacteriol 171:3102–3107.
59. Schut G, Nixon W, Lipscomb G, Scott R, Adams M. 2012. Mutational analyses of the enzymes involved in the metabolism of hydrogen by the hyperthermophilic archaeon *Pyrococcus furiosus*. Front Microbiol 3:163. https://doi.org/10.3389/fmicb.2012.00163.
60. Welte C, Krätzer C, Deppenmeier U. 2010. Involvement of Ech hydrogenase in energy conservation of *Methanosarcina mazei*. FEBS J 277:3396–3403. https://doi.org/10.1111/j.1742-4658.2010.07744.x.
61. Schut GJ, Boyd ES, Peters JW, Adams MW. 2013. The modular respiratory complexes involved in hydrogen and sulfur metabolism by heterotrophic hyperthermophilic archaea and their evolutionary implications. FEMS Microbiol Rev 37:182–203. https://doi.org/10.1111/j.1574-6976.2012.00346.x.
62. Schäfer C, Friedrich B, Lenz O. 2013. Novel, oxygen-insensitive group 5 [NiFe]-hydrogenase in *Ralstonia eutropha*. Appl Environ Microbiol 79:5137–5145. https://doi.org/10.1128/AEM.01576-13.
63. Constant P, Chowdhury SP, Pratscher J, Conrad R. 2010. Streptomycetes contributing to atmospheric molecular hydrogen soil uptake are widespread and encode a putative high-affinity [NiFe]-hydrogenase. Environ Microbiol 12:821–829. https://doi.org/10.1111/j.1462-2920.2009.02130.x.
64. Constant P, Poissant L, Villemur R. 2008. Isolation of *Streptomyces* sp. PCB7, the first microorganism demonstrating high-affinity uptake of tropospheric H_2. ISME J 2:1066–1076. https://doi.org/10.1038/ismej.2008.59.
65. Greening C, Berney M, Hards K, Cook GM, Conrad R. 2014. A soil actinobacterium scavenges atmospheric H_2 using two membrane-associated, oxygen-dependent [NiFe] hydrogenases. Proc Natl Acad Sci U S A 111:4257–4261. https://doi.org/10.1073/pnas.1320586111.
66. Constant P, Chowdhury SP, Hesse L, Pratscher J, Conrad R. 2011. Genome data mining and soil survey for the novel group 5 [NiFe]-hydrogenase to explore the diversity and ecological importance of presumptive high-affinity H_2-oxidizing bacteria. Appl Environ Microbiol 77:6027–6035. https://doi.org/10.1128/AEM.00673-11.
67. Greening C, Villas-Boas SG, Robson JR, Berney M, Cook GM. 2014. The growth and survival of *Mycobacterium smegmatis* is enhanced by co-metabolism of atmospheric H_2. PLoS One 9:e103034. https://doi.org/10.1371/journal.pone.0103034.
68. Liot Q, Constant P. 2016. Breathing air to save energy–new insights into the ecophysiological role of high-affinity [NiFe]-hydrogenase in *Streptomyces avermitilis*. Microbiologyopen 5:47–59. https://doi.org/10.1002/mbo3.310.
69. Schwartz E, Henne A, Cramm R, Eitinger T, Friedrich B, Gottschalk G. 2003. Complete nucleotide sequence of pHG1: a *Ralstonia eutropha* H16 megaplasmid encoding key enzymes of H_2-based lithoautotrophy and anaerobiosis. J Mol Biol 332:369–383. https://doi.org/10.1016/S0022-2836(03)00894-5.
70. Schäfer C, Bommer M, Hennig SE, Jeoung J-H, Dobbek H, Lenz O. 2016. Structure of an actinobacterial-type [NiFe]-hydrogenase reveals insight into O_2-tolerant H_2 oxidation. Structure 24:285–292. https://doi.org/10.1016/j.str.2015.11.010.
71. Piché-Choquette S, Khdhiri M, Constant P. 2017. Survey of high-affinity H_2-oxidizing bacteria in soil reveals their vast diversity yet underrepresentation in genomic databases. Microb Ecol 74:771–775. https://doi.org/10.1007/s00248-017-1011-1.
72. Gaffron H, Rubin J. 1942. Fermentative and photochemical production of hydrogen in algae. J Gen Physiol 26:219–240.
73. Yagi T, Yamashita K, Okada N, Isono T, Momose D, Mineki S, Tokunaga E. 2016. Hydrogen photoproduction in green algae *Chlamydomonas reinhardtii* sustainable over 2 weeks with the original cell culture without supply of fresh cells nor exchange of the whole culture medium. J Plant Res 129:771–779. https://doi.org/10.1007/s10265-016-0825-0.
74. Tsygankov AA, Fedorov AS, Laurinavichene TV, Gogotov IN, Rao KK, Hall DO. 1998. Actual and potential rates of hydrogen photoproduction by continuous culture of the purple non-sulphur bacterium *Rhodobacter capsulatus*. Appl Microbiol Biotechnol 49:102–107. https://doi.org/10.1007/s002530051144.
75. Oh Y-K, Seol E-H, Kim M-S, Park S. 2004. Photoproduction of hydrogen

from acetate by a chemoheterotrophic bacterium *Rhodopseudomonas palustris* P4. Int J Hydrogen Energy 29:1115–1121. https://doi.org/10.1016/j.ijhydene.2003.11.008.
76. Ghirardi ML, Zhang L, Lee JW, Flynn T, Seibert M, Greenbaum E, Melis A. 2000. Microalgae: a green source of renewable H_2. Trends Biotechnol 18:506–511. https://doi.org/10.1016/S0167-7799(00)01511-0.
77. Benemann JR. 2000. Hydrogen production by microalgae. J Appl Phycol 12:291–300. https://doi.org/10.1023/A:1008175112704.
78. Bourke MF, Marriott PJ, Glud RN, Hasler-Sheetal H, Kamalanathan M, Beardall J, Greening C, Cook PLM. 2017. Metabolism in anoxic permeable sediments is dominated by eukaryotic dark fermentation. Nat Geosci 10:30–35. https://doi.org/10.1038/ngeo2843.
79. Zhang H, Bruns MA, Logan BE. 2006. Biological hydrogen production by *Clostridium acetobutylicum* in an unsaturated flow reactor. Water Res 40:728–734. https://doi.org/10.1016/j.watres.2005.11.041.
80. Redwood MD, Paterson-Beedle M, Macaskie LE. 2009. Integrating dark and light bio-hydrogen production strategies: towards the hydrogen economy. Rev Environ Sci Biotechnol 8:149. https://doi.org/10.1007/s11157-008-9144-9.
81. Calusinska M, Happe T, Joris B, Wilmotte A. 2010. The surprising diversity of clostridial hydrogenases: a comparative genomic perspective. Microbiology 156:1575–1588. https://doi.org/10.1099/mic.0.032771-0.
82. Schut GJ, Adams MW. 2009. The iron-hydrogenase of *Thermotoga maritima* utilizes ferredoxin and NADH synergistically: a new perspective on anaerobic hydrogen production. J Bacteriol 191:4451–4457. https://doi.org/10.1128/JB.01582-08.
83. Khanna N, Das D. 2013. Biohydrogen production by dark fermentation. Wiley Interdiscip Rev Energy Environ 2:401–421. https://doi.org/10.1002/wene.15.
84. Dong X, Greening C, Brüls T, Conrad R, Guo K, Blaskowski S, Kaschani F, Kaiser M, Laban NA, Meckenstock RU. 2018. Fermentative Spirochaetes mediate necromass recycling in anoxic hydrocarbon-contaminated habitats. ISME J 12:2039–2050. https://doi.org/10.1038/s41396-018-0148-3.
85. Mei N, Postec A, Monnin C, Pelletier B, Payri CE, Ménez B, Frouin E, Ollivier B, Erauso G, Quéméneur M. 2016. Metagenomic and PCR-based diversity surveys of [FeFe]-hydrogenases combined with isolation of alkaliphilic hydrogen-producing bacteria from the serpentinite-hosted Prony Hydrothermal Field, New Caledonia. Front Microbiol 7:1301. https://doi.org/10.3389/fmicb.2016.01301.
86. Boyd ES, Hamilton TL, Swanson KD, Howells AE, Baxter BK, Meuser JE, Posewitz MC, Peters JW. 2014. [FeFe]-hydrogenase abundance and diversity along a vertical redox gradient in Great Salt Lake, USA. Int J Mol Sci 15:21947–21966. https://doi.org/10.3390/ijms151221947.
87. Baba R, Kimura M, Asakawa S, Watanabe T. 2014. Analysis of [FeFe]-hydrogenase genes for the elucidation of a hydrogen-producing bacterial community in paddy field soil. FEMS Microbiol Lett 350:249–256. https://doi.org/10.1111/1574-6968.12335.
88. Baba R, Asakawa S, Watanabe T. 2016. H_2-producing bacterial community during rice straw decomposition in paddy field soil: estimation by an analysis of [FeFe]-hydrogenase gene transcripts. Microbes Environ 31:226–233. https://doi.org/10.1264/jsme2.ME16036.
89. Schmidt O, Drake HL, Horn MA. 2010. Hitherto unknown [FeFe]-hydrogenase gene diversity in anaerobes and anoxic enrichments from a moderately acidic fen. Appl Environ Microbiol 76:2027–2031. https://doi.org/10.1128/AEM.02895-09.
90. Leigh JA. 2000. Nitrogen fixation in methanogens: the archaeal perspective. Curr Issues Mol Biol 2:125–131.
91. Orr CH, James A, Leifert C, Cooper JM, Cummings SP. 2011. Diversity and activity of free-living nitrogen-fixing bacteria and total bacteria in organic and conventionally managed soils. Appl Environ Microbiol 77:911–919. https://doi.org/10.1128/AEM.01250-10.
92. Fowler D, Coyle M, Skiba U, Sutton MA, Cape JN, Reis S, Sheppard LJ, Jenkins A, Grizzetti B, Galloway JN, Vitousek P, Leach A, Bouwman AF, Butterbach-Bahl K, Dentener F, Stevenson D, Amann M, Voss M. 2013. The global nitrogen cycle in the twenty-first century. Philos Trans R Soc Lond B Biol Sci 368:20130165. https://doi.org/10.1098/rstb.2013.0164.
93. Jayakumar A, Chang BX, Widner B, Bernhardt P, Mulholland MR, Ward BB. 2017. Biological nitrogen fixation in the oxygen-minimum region of the eastern tropical North Pacific ocean. ISME J 11:2356. https://doi.org/10.1038/ismej.2017.97.
94. Voss M, Bange HW, Dippner JW, Middelburg JJ, Montoya JP, Ward B. 2013. The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change. Philos Trans R Soc Lond B Biol Sci 368:20130121. https://doi.org/10.1098/rstb.2013.0121.
95. Vitousek PM, Menge DNL, Reed SC, Cleveland CC. 2013. Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Philos Trans R Soc Lond B Biol Sci 368:20130119. https://doi.org/10.1098/rstb.2013.0119.
96. Hunt S, Gaito S, Layzell D. 1988. Model of gas exchange and diffusion in legume nodules. II: Characterisation of the diffusion barrier and estimation of the concentrations of CO_2, H_2 and N_2 in the infected cells. Planta 173:128–141. https://doi.org/10.1007/BF00394497.
97. Postgate JR. 1982. Biology nitrogen fixation: fundamentals. Philos Trans R Soc Lond B Biol Sci 296:375–385. https://doi.org/10.1098/rstb.1982.0013.
98. Burris RH. 1991. Nitrogenases. J Biol Chem 266:9339–9342.
99. Mylona P, Pawlowski K, Bisseling T. 1995. Symbiotic nitrogen fixation. Plant Cell 7:869–885. https://doi.org/10.1105/tpc.7.7.869.
100. Berman-Frank I, Lundgren P, Chen Y-B, Küpper H, Kolber Z, Bergman B, Falkowski P. 2001. Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium *Trichodesmium*. Science 294:1534–1537. https://doi.org/10.1126/science.1064082.
101. Brewin NJ. 1991. Development of the legume root nodule. Annu Rev Cell Biol 7:191–226. https://doi.org/10.1146/annurev.cb.07.110191.001203.
102. Stal LJ, Krumbein WE. 1987. Temporal separation of nitrogen fixation and photosynthesis in the filamentous, non-heterocystous cyanobacterium *Oscillatoria* sp. Arch Microbiol 149:76–80. https://doi.org/10.1007/BF00423140.
103. Poole RK, Hill S. 1997. Respiratory protection of nitrogenase activity in *Azotobacter vinelandii*—roles of the terminal oxidases. Biosci Rep 17:303–317.
104. Hunt S, Layzell DB. 1993. Gas exchange of legume nodules and the regulation of nitrogenase activity. Annu Rev Plant Physiol Plant Mol Biol 44:483–511. https://doi.org/10.1146/annurev.pp.44.060193.002411.
105. Witty JF. 1991. Microelectrode measurements of hydrogen concentrations and gradients in legume nodules. J Exp Bot 42:765–771. https://doi.org/10.1093/jxb/42.6.765.
106. Witty JF, Minchin FR. 1998. Hydrogen measurements provide direct evidence for a variable physical barrier to gas diffusion in legume nodules. J Exp Bot 49:1015–1020. https://doi.org/10.1093/jxb/49.323.1015.
107. Dixon ROD. 1972. Hydrogenase in legume root nodule bacteroids: occurrence and properties. Arch Mikrobiol 85:193–201.
108. Mus F, Crook MB, Garcia K, Garcia Costas A, Geddes BA, Kouri ED, Paramasivan P, Ryu M-H, Oldroyd GED, Poole PS, Udvardi MK, Voigt CA, Ané J-M, Peters JW. 2016. Symbiotic nitrogen fixation and the challenges to its extension to non-legumes. Appl Environ Microbiol 82:3698–3710. https://doi.org/10.1128/AEM.01055-16.
109. Steenhoudt O, Vanderleyden J. 2000. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol Rev 24:487–506. https://doi.org/10.1111/j.1574-6976.2000.tb00552.x.
110. Tschapek M, Giambiagi N. 1955. Nitrogen fixation of *Azotobacter* in soil—its inhibition by oxygen. Arch Mikrobiol 21:376–390.
111. Uratsu SL, Keyser HH, Weber DF, Lim ST. 1982. Hydrogen uptake (Hup) activity of *Rhizobium japonicum* from major United-States soybean production areas. Crop Sci 22:600–602. https://doi.org/10.2135/cropsci1982.0011183X002200030040x.
112. Maimaiti J, Zhang Y, Yang J, Cen Y-P, Layzell DB, Peoples M, Dong Z. 2007. Isolation and characterization of hydrogen-oxidizing bacteria induced following exposure of soil to hydrogen gas and their impact on plant growth. Environ Microbiol 9:435–444. https://doi.org/10.1111/j.1462-2920.2006.01155.x.
113. Dong Z, Wu L, Kettlewell B, Caldwell CD, Layzell DB. 2003. Hydrogen fertilization of soils–is this a benefit of legumes in rotation? Plant Cell Environ 26:1875–1879. https://doi.org/10.1046/j.1365-3040.2003.01103.x.
114. He Y, Li M, Perumal V, Feng X, Fang J, Xie J, Sievert SM, Wang F. 2016. Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum Bathyarchaeota widespread in marine sediments. Nat Microbiol 1:16035. https://doi.org/10.1038/nmicrobiol.2016.35.
115. Rosencrantz D, Rainey FA, Janssen PH. 1999. Culturable populations of Sporomusa spp. and Desulfovibrio spp. in the anoxic bulk soil of flooded rice microcosms. Appl Environ Microbiol 65:3526–3533.

116. Breznak JA. 1994. Acetogenesis from carbon dioxide in termite guts, p 303–330. *In* Drake HL (ed), Acetogenesis. Springer US, Boston, MA.
117. Rey FE, Faith JJ, Bain J, Muehlbauer MJ, Stevens RD, Newgard CB, Gordon JI. 2010. Dissecting the in vivo metabolic potential of two human gut acetogens. J Biol Chem 285:22082–22090. https://doi.org/10.1074/jbc.M110.117713.
118. Liu S, Suflita JM. 1993. H_2-CO_2-dependent anaerobic O-demethylation activity in subsurface sediments and by an isolated bacterium. Appl Environ Microbiol 59:1325–1331.
119. Ollivier B, Caumette P, Garcia JL, Mah RA. 1994. Anaerobic bacteria from hypersaline environments. Microbiol Rev 58:27–38.
120. Peters V, Conrad R. 1995. Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. Appl Environ Microbiol 61:1673–1676.
121. Karnholz A, Küsel K, Gössner A, Schramm A, Drake HL. 2002. Tolerance and metabolic response of acetogenic bacteria toward oxygen. Appl Environ Microbiol 68:1005–1009. https://doi.org/10.1128/AEM.68.2.1005-1009.2002.
122. Küsel K, Karnholz A, Trinkwalter T, Devereux R, Acker G, Drake HL. 2001. Physiological ecology of Clostridium glycolicum RD-1, an aerotolerant acetogen isolated from sea grass roots. Appl Environ Microbiol 67:4734–4741. https://doi.org/10.1128/AEM.67.10.4734-4741.2001.
123. Ragsdale SW, Pierce E. 2008. Acetogenesis and the Wood–Ljungdahl pathway of CO_2 fixation. Biochim Biophys Acta 1784:1873–1898. https://doi.org/10.1016/j.bbapap.2008.08.012.
124. Fischer F, Lieske R, Winzer K. 1932. Biologische Gasreaktionen. II. Über die Bildung von Essigsäure bei der biologischen Umsetzung von Kohlenoxyd und Kohlensäure mit Wasserstoff zu Methan. Biochem Z 245:2–12.
125. le Van TD, Robinson JA, Ralph J, Greening RC, Smolenski WJ, Leedle JAZ, Schaefer DM. 1998. Assessment of reductive acetogenesis with indigenous ruminal bacterium populations and Acetitomaculum ruminis. Appl Environ Microbiol 64:3429–3436.
126. Schink B. 1997. Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 61:262–280.
127. Pester M, Brune A. 2007. Hydrogen is the central free intermediate during lignocellulose degradation by termite gut symbionts. ISME J 1:551–565. https://doi.org/10.1038/ismej.2007.62.
128. Liou JS-C, Balkwill DL, Drake GR, Tanner RS. 2005. *Clostridium carboxidivorans* sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen *Clostridium scatologenes* strain SL1 as *Clostridium drakei* sp. nov. Int J Syst Evol Microbiol 55:2085–2091. https://doi.org/10.1099/ijs.0.63482-0.
129. Küsel K, Dorsch T, Acker G, Stackebrandt E, Drake HL. 2000. *Clostridium scatologenes* strain SL1 isolated as an acetogenic bacterium from acidic sediments. Int J Syst Evol Microbiol 50:537–546. https://doi.org/10.1099/00207713-50-2-537.
130. Koeck DE, Pechtl A, Zverlov VV, Schwarz WH. 2014. Genomics of cellulolytic bacteria. Curr Opin Biotechnol 29:171–183. https://doi.org/10.1016/j.copbio.2014.07.002.
131. Ueno A, Shimizu S, Tamamura S, Okuyama H, Naganuma T, Kaneko K. 2016. Anaerobic decomposition of humic substances by *Clostridium* from the deep subsurface. Sci Rep 6:18990. https://doi.org/10.1038/srep18990.
132. Conrad R. 2009. The global methane cycle: recent advances in understanding the microbial processes involved. Environ Microbiol Rep 1:285–292. https://doi.org/10.1111/j.1758-2229.2009.00038.x.
133. Thauer RK. 1998. Biochemistry of methanogenesis: a tribute to Marjory Stephenson:1998 Marjory Stephenson Prize Lecture. Microbiology 144:2377–2406. https://doi.org/10.1099/00221287-144-9-2377.
134. Stephenson M, Stickland LH. 1933. Hydrogenase: the bacterial formation of methane by the reduction of one-carbon compounds by molecular hydrogen. Biochem J 27:1517–1527.
135. Jabłoński S, Rodowicz P, Łukaszewicz M. 2015. Methanogenic archaea database containing physiological and biochemical characteristics. Int J Syst Evol Microbiol 65:1360–1368. https://doi.org/10.1099/ijs.0.000065.
136. Singh N, Kendall MM, Liu Y, Boone DR. 2005. Isolation and characterization of methylotrophic methanogens from anoxic marine sediments in Skan Bay, Alaska: description of *Methanococcoides alaskense* sp. nov., and emended description of *Methanosarcina baltica*. Int J Syst Evol Microbiol 55:2531–2538. https://doi.org/10.1099/ijs.0.63886-0.
137. Costa KC, Lie TJ, Jacobs MA, Leigh JA. 2013. H_2-independent growth of the hydrogenotrophic methanogen Methanococcus maripaludis. mBio 4:e00062-13. https://doi.org/10.1128/mBio.00062-13.
138. Angle JC, Morin TH, Solden LM, Narrowe AB, Smith GJ, Borton MA, Rey-Sanchez C, Daly RA, Mirfenderesgi G, Hoyt DW, Riley WJ, Miller CS, Bohrer G, Wrighton KC. 2017. Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions. Nat Commun 8:1567. https://doi.org/10.1038/s41467-017-01753-4.
139. Bond DR, Lovley DR. 2002. Reduction of Fe(III) oxide by methanogens in the presence and absence of extracellular quinones. Environ Microbiol 4:115–124. https://doi.org/10.1046/j.1462-2920.2002.00279.x.
140. Bertram S, Blumenberg M, Michaelis W, Siegert M, Krüger M, Seifert R. 2013. Methanogenic capabilities of ANME-archaea deduced from ^{13}C-labelling approaches. Environ Microbiol 15:2384–2393. https://doi.org/10.1111/1462-2920.12112.
141. Timmers PHA, Welte CU, Koehorst JJ, Plugge CM, Jetten MSM, Stams AJM. 2017. Reverse methanogenesis and respiration in methanotrophic archaea. Archaea 2017:1654237. https://doi.org/10.1155/2017/1654237.
142. Thauer RK, Kunow J. 1995. Sulfate-reducing archaea, p 33–48. *In* Barton LL (ed), Sulfate-reducing bacteria. Springer US, Boston, MA.
143. Muyzer G, Stams AJM. 2008. The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6:441. https://doi.org/10.1038/nrmicro1892.
144. Jørgensen BB. 1977. The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark). Limnol Oceanogr 22:814–832. https://doi.org/10.4319/lo.1977.22.5.0814.
145. Cervantes FJ, de Bok FA, Duong-Dac T, Stams AJ, Lettinga G, Field JA. 2002. Reduction of humic substances by halorespiring, sulphate-reducing and methanogenic microorganisms. Environ Microbiol 4:51–57. https://doi.org/10.1046/j.1462-2920.2002.00258.x.
146. Gorny N, Schink B. 1994. Anaerobic degradation of catechol by *Desulfobacterium* sp. strain Cat2 proceeds via carboxylation to protocatechuate. Appl Environ Microbiol 60:3396–3400.
147. Kim SH, Harzman C, Davis JK, Hutcheson R, Broderick JB, Marsh TL, Tiedje JM. 2012. Genome sequence of *Desulfitobacterium hafniense* DCB-2, a Gram-positive anaerobe capable of dehalogenation and metal reduction. BMC Microbiol 12:21. https://doi.org/10.1186/1471-2180-12-21.
148. Schauder R, Widdel F, Fuchs G. 1987. Carbon assimilation pathways in sulfate-reducing bacteria II. Enzymes of a reductive citric acid cycle in the autotrophic *Desulfobacter hydrogenophilus*. Arch Microbiol 148:218–225. https://doi.org/10.1007/BF00414815.
149. Parshina SN, Sipma J, Henstra AM, Stams AJM. 2010. Carbon monoxide as an electron donor for the biological reduction of sulphate. Int J Microbiol 2010:319527. https://doi.org/10.1155/2010/319527.
150. Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, Gieseke A, Amann R, Jørgensen BB, Witte U, Pfannkuche O. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623. https://doi.org/10.1038/35036572.
151. Das A, Mishra AK, Roy P. 1992. Anaerobic growth on elemental sulfur using dissimilar iron reduction by autotrophic *Thiobacillus ferrooxidans*. FEMS Microbiol Lett 97:167–172. https://doi.org/10.1111/j.1574-6968.1992.tb05457.x.
152. Lonergan DJ, Jenter HL, Coates JD, Phillips EJ, Schmidt TM, Lovley DR. 1996. Phylogenetic analysis of dissimilatory Fe(III)-reducing bacteria. J Bacteriol 178:2402–2408.
153. Kashefi K, Tor JM, Holmes DE, Gaw Van Praagh CV, Reysenbach AL, Lovley DR. 2002. *Geoglobus ahangari* gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the sole electron acceptor. Int J Syst Evol Microbiol 52:719–728. https://doi.org/10.1099/00207713-52-3-719.
154. Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedy DW, Pinchuk G, Watanabe K, Ishii S, Logan B, Nealson KH, Fredrickson JK. 2006. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. Proc Natl Acad Sci U S A 103:11358–11363. https://doi.org/10.1073/pnas.0604517103.
155. Lovley DR, Holmes DE, Nevin KP. 2004. Dissimilatory Fe(III) and Mn(IV) reduction. Adv Microb Physiol 49:219–286. https://doi.org/10.1016/S0065-2911(04)49005-5.
156. Thauer RK, Jungermann K, Decker K. 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180.

157. Lovley DR. 1993. Dissimilatory metal reduction. Annu Rev Microbiol 47:263–290. https://doi.org/10.1146/annurev.mi.47.100193.001403.
158. Kashefi K, Lovley DR. 2000. Reduction of Fe(III), Mn(IV), and toxic metals at 100°C by *Pyrobaculum islandicum*. Appl Environ Microbiol 66:1050–1056. https://doi.org/10.1128/AEM.66.3.1050-1056.2000.
159. Lovley DR, Goodwin S. 1988. Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments. Geochim Cosmochim Acta 52:2993–3003. https://doi.org/10.1016/0016-7037(88)90163-9.
160. Lovley DR, Coates JD, Blunt-Harris EL, Phillips EJP, Woodward JC. 1996. Humic substances as electron acceptors for microbial respiration. Nature 382:445. https://doi.org/10.1038/382445a0.
161. Lee K-C, Rittmann BE. 2002. Applying a novel autohydrogenotrophic hollow-fiber membrane biofilm reactor for denitrification of drinking water. Water Res 36:2040–2052. https://doi.org/10.1016/S0043-1354(01)00425-0.
162. Ontiveros-Valencia A, Ilhan ZE, Kang D-W, Rittmann B, Krajmalnik-Brown R. 2013. Phylogenetic analysis of nitrate- and sulfate-reducing bacteria in a hydrogen-fed biofilm. FEMS Microbiol Ecol 85:158–167. https://doi.org/10.1111/1574-6941.12107.
163. Seitzinger S, Harrison JA, Böhlke JK, Bouwman AF, Lowrance R, Peterson B, Tobias C, Drecht GV. 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecol Appl 16:2064–2090.
164. Zumft WG. 1997. Cell biology and molecular basis of denitrification. Microbiol Mol Biol Rev 61:533–616.
165. Simon J, Klotz MG. 2013. Diversity and evolution of bioenergetic systems involved in microbial nitrogen compound transformations. Biochim Biophys Acta 1827:114–135. https://doi.org/10.1016/j.bbabio.2012.07.005.
166. Tian J-H, Pourcher A-M, Bouchez T, Gelhaye E, Peu P. 2014. Occurrence of lignin degradation genotypes and phenotypes among prokaryotes. Appl Microbiol Biotechnol 98:9527–9544. https://doi.org/10.1007/s00253-014-6142-4.
167. Iyer R, Iken B, Damania A. 2017. Genome of *Pseudomonas nitroreducens* DF05 from dioxin contaminated sediment downstream of the San Jacinto River waste pits reveals a broad array of aromatic degradation gene determinants. Genom Data 14:40–43. https://doi.org/10.1016/j.gdata.2017.07.011.
168. Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, Smolders AJP, Ettwig KF, Rijpstra WIC, Schouten S, Damsté JSS, Op den Camp HJM, Jetten MSM, Strous M. 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440:918–921. https://doi.org/10.1038/nature04617.
169. Fritsch J, Lenz O, Friedrich B. 2013. Structure, function and biosynthesis of O_2-tolerant hydrogenases. Nat Rev Microbiol 11:106–114. https://doi.org/10.1038/nrmicro2940.
170. Rasche ME, Arp DJ. 1989. Hydrogen inhibition of nitrogen reduction by nitrogenase in isolated soybean nodule bacteroids. Plant Physiol 91:663–668.
171. Golding AL, Zou Y, Yang X, Flynn B, Dong Z. 2012. Plant growth promoting H_2-oxidizing bacteria as seed inoculants for cereal crops. Agric Sci 3:510–516. https://doi.org/10.4236/as.2012.34060.
172. Ma C, Zhuang L, Zhou SG, Yang GQ, Yuan Y, Xu RX. 2012. Alkaline extracellular reduction: isolation and characterization of an alkaliphilic and halotolerant bacterium, *Bacillus pseudofirmus* MC02. J Appl Microbiol 112:883–891. https://doi.org/10.1111/j.1365-2672.2012.05276.x.
173. Yang G, Zhou X, Zhou S, Yang D, Wang Y, Wang D. 2013. *Bacillus thermotolerans* sp. nov., a thermophilic bacterium capable of reducing humus. Int J Syst Evol Microbiol 63:3672–3678. https://doi.org/10.1099/ijs.0.048942-0.
174. Mohammadi S, Pol A, van Alen TA, Jetten MSM, Op den Camp HJM. 2017. *Methylacidiphilum fumariolicum* SolV, a thermoacidophilic 'Knallgas' methanotroph with both an oxygen-sensitive and -insensitive hydrogenase. ISME J 11:945–958. https://doi.org/10.1038/ismej.2016.171.
175. Chen Y, Yoch D. 1987. Regulation of two nickel-requiring (inducible and constitutive) hydrogenases and their coupling to nitrogenase in *Methylosinus trichosporium* OB3b. J Bacteriol 169:4778–4783.
176. Bergmann FH, Towne JC, Burris RH. 1958. Assimilation of carbon dioxide by hydrogen bacteria. J Biol Chem 230:13–24.
177. Bowien B, Kusian B. 2002. Genetics and control of CO_2 assimilation in the chemoautotroph *Ralstonia eutropha*. Arch Microbiol 178:85–93. https://doi.org/10.1007/s00203-002-0441-3.
178. Weber CF, King GM. 2012. The phylogenetic distribution and ecological role of carbon monoxide oxidation in the genus *Burkholderia*. FEMS Microbiol Ecol 79:167–175. https://doi.org/10.1111/j.1574-6941.2011.01206.x.
179. Guo R, Conrad R. 2008. Extraction and characterization of soil hydrogenases oxidizing atmospheric hydrogen. Soil Biol Biochem 40:1149–1154. https://doi.org/10.1016/j.soilbio.2007.12.007.
180. Conrad R, Aragno M, Seiler W. 1983. The inability of hydrogen bacteria to utilize atmospheric hydrogen is due to threshold and affinity for hydrogen. FEMS Microbiol Lett 18:207–210. https://doi.org/10.1111/j.1574-6968.1983.tb00479.x.
181. Meredith LK, Rao D, Bosak T, Klepac-Ceraj V, Tada KR, Hansel CM, Ono S, Prinn RG. 2014. Consumption of atmospheric hydrogen during the life cycle of soil-dwelling actinobacteria. Environ Microbiol Rep 6:226–238. https://doi.org/10.1111/1758-2229.12116.
182. Pieterse G, Krol MC, Batenburg AM, M Brenninkmeijer CA, Popa ME, O'Doherty S, Grant A, Steele LP, Krummel PB, Langenfelds RL, Wang HJ, Vermeulen AT, Schmidt M, Yver C, Jordan A, Engel A, Fisher RE, Lowry D, Nisbet EG, Reimann S, Vollmer MK, Steinbacher M, Hammer S, Forster G, Sturges WT, Röckmann T. 2013. Reassessing the variability in atmospheric H_2 using the two-way nested TM5 model. J Geophys Res Atmos 118:3764–3780. https://doi.org/10.1002/jgrd.50204.
183. Janusz G, Pawlik A, Sulej J, Swiderska-Burek U, Jarosz-Wilkolazka A, Paszczynski A. 2017. Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution. FEMS Microbiol Rev 41:941–962. https://doi.org/10.1093/femsre/fux049.
184. Jaouadi B, Rekik H, Badis A, Jaouadi NZ, Belhoul M, Hmidi M, Kourdali S, Fodil D, Bejar S. 2014. Production, purification, and characterization of a highly thermostable and humic acid biodegrading peroxidase from a decolorizing *Streptomyces albidoflavus* strain TN644 isolated from a Tunisian off-shore oil field. Int Biodeterior Biodegradation 90:36–44. https://doi.org/10.1016/j.ibiod.2014.02.001.
185. Fodil D, Badis A, Jaouadi B, Zaraî N, Ferradji FZ, Boutoumi H. 2011. Purification and characterization of two extracellular peroxidases from *Streptomyces* sp. strain AM2, a decolorizing actinomycetes responsible for the biodegradation of natural humic acids. Int Biodeterior Biodegradation 65:470–478. https://doi.org/10.1016/j.ibiod.2011.01.009.
186. Quigley DR, Ward B, Crawford DL, Hatcher HJ, Dugan PR. 1989. Evidence that microbially produced alkaline materials are involved in coal biosolubilization. Appl Biochem Biotechnol 20:753–763. https://doi.org/10.1007/BF02936523.
187. King GM. 2003. Uptake of carbon monoxide and hydrogen at environmentally relevant concentrations by mycobacteria. Appl Environ Microbiol 69:7266–7272. https://doi.org/10.1128/AEM.69.12.7266-7272.2003.
188. Ji M, Greening C, Vanwonterghem I, Carere CR, Bay SK, Steen JA, Montgomery K, Lines T, Beardall J, Van Dorst J, Snape I, Stott MB, Hugenholtz P, Ferrari BC. 2017. Atmospheric trace gases support primary production in Antarctic desert surface soil. Nature 552:400–403. https://doi.org/10.1038/nature25014.
189. Martinez CM, Alvarez LH, Celis LB, Cervantes FJ. 2013. Humus-reducing microorganisms and their valuable contribution in environmental processes. Appl Microbiol Biotechnol 97:10293–10308. https://doi.org/10.1007/s00253-013-5350-7.
190. Khdhiri M, Piché-Choquette S, Tremblay J, Tringe SG, Constant P. 2017. The tale of a neglected energy source: elevated hydrogen exposure affects both microbial diversity and function in soil. Appl Environ Microbiol 83:e00275-17. https://doi.org/10.1128/AEM.00275-17.
191. Piché-Choquette S, Tremblay J, Tringe SG, Constant P. 2016. H_2-saturation of high affinity H_2-oxidizing bacteria alters the ecological niche of soil microorganisms unevenly among taxonomic groups. PeerJ 4:e1782. https://doi.org/10.7717/peerj.1782.
192. Yonemura S, Kawashima S, Tsuruta H. 1999. Continuous measurements of CO and H_2 deposition velocities onto an andisol: uptake control by soil moisture. Tellus B Chem Phys Meteorol 51:688–700. https://doi.org/10.3402/tellusb.v51i3.16465.
193. Yonemura S, Kawashima S, Tsuruta H. 2000. Carbon monoxide, hydrogen, and methane uptake by soils in a temperate arable field and a forest. J Geophys Res 105:14347–14362. https://doi.org/10.1029/1999JD901156.
194. Piché-Choquette S, Khdhiri M, Constant P. 2018. Dose-response relationships between environmentally-relevant H_2 concentrations and the biological sinks of H_2, CH_4 and CO in soil. Soil Biol Biochem 123:190–199. https://doi.org/10.1016/j.soilbio.2018.05.008.

195. Dong Z, Layzell DB. 2001. H_2 oxidation, O_2 uptake and CO_2 fixation in hydrogen treated soils. Plant Soil 229:1–12.
196. Stein S, Selesi D, Schilling R, Pattis I, Schmid M, Hartmann A. 2005. Microbial activity and bacterial composition of H_2-treated soils with net CO_2 fixation. Soil Biol Biochem 37:1938–1945. https://doi.org/10.1016/j.soilbio.2005.02.035.
197. Bhowmik A, Cloutier M, Ball E, Bruns MA. 2017. Underexplored microbial metabolisms for enhanced nutrient recycling in agricultural soils. AIMS Microbiol 3:826–845. https://doi.org/10.3934/microbiol.2017.4.826.
198. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340.
199. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033.
200. Swofford DL. 2003. Phylogenetic Analysis Using Parsimony, version 4. Sinauer Associates, Sunderland, MA.
201. Gascuel O. 1997. BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. Mol Biol Evol 14:685–695. https://doi.org/10.1093/oxfordjournals.molbev.a025808.
202. Miller MA, Pfeiffer W, Schwartz T. 2011. The CIPRES science gateway: a community resource for phylogenetic analyses, article 41. Proceedings of the 2011 TeraGrid Conference: Extreme Digital Discovery, 18 to 21 July 2011, Salt Lake City, UT.
203. Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290. https://doi.org/10.1093/bioinformatics/btg412.
204. R Development Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.