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REVIEW PAPER

Aerobic hydrogen-oxidizing bacteria in soil: from cells to ecosystems

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Abstract Aerobic hydrogen-oxidizing bacteria (HOB) is a group of active, abundant, and diverse microorganisms with representatives in nearly all phyla, distributed in soil exposed to atmospheric or elevated hydrogen. In this review, first, we discuss the fundamental physiology, isolation, and identification techniques for HOB. On this basis, the hydrogenase genetic organization and metabolic strategy of *Cupriavidus necator*, *Mycobacterium smegmatis* as representative HOB are summarized. Availability of hydrogen, oxygen, nutrients, and environmental variables such as temperature and moisture are key ecological factors influencing H_2 oxidation activity, hydrogenase groups, and microbial composition. Finally, we systematically illustrate the ecological roles of HOB and the interactions between HOB and other soil microbiota, particularly rhizobia in agricultural soils and *Cyanobacteria* in deserts. Intensive studies should focus on the cell functioning regulated by hydrogenases and on the full play of ecological roles by competitive HOB consortiums in soil niches, which is expected to facilitate the biogeochemical process of carbon dioxide fixation and energy utilization.

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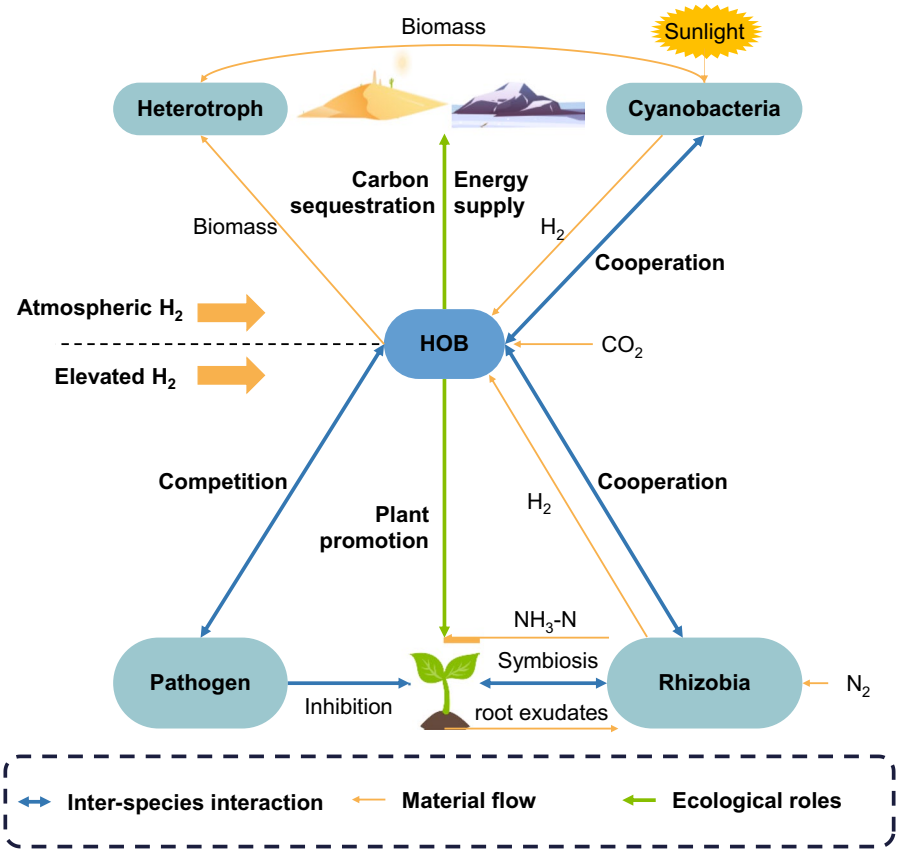
Graphical abstract



Keywords Carbon fixation · Hydrogen metabolism · Hydrogenase · Soil ecology · Inter-species interaction

1 Introduction

The total amount of molecular hydrogen (H_2) released annually into the troposphere has been estimated to be 107 ± 15 Tg (Rhee et al. 2006; Schwartz et al. 2013). The consumption of atmospheric H_2 is dominated by the uptake in soils, which accounts for over 90% of the global H_2 sink (Schwartz et al. 2013). Remarkably, consumption of atmospheric H_2 in soils may be to a significant extent indirectly attributable to aerobic hydrogen oxidizing bacteria (HOB) (Schwartz et al. 2013). Aerobic HOB that utilize H_2 as an electron donor and O_2 as an electron acceptor is a physiological group with representatives in nearly all phyla (Pumphrey et al. 2011; Yu 2018; Ehsani et al. 2019). The key enzymes which catalyze the oxidation of H_2 into protons and electrons are hydrogenases in HOB.

The HOB group occurs ubiquitously in soil niches with elevated levels of H_2 in agricultural soil, volcanic or geothermal sites, and termite mounds. HOB in H_2-enriched soils are often facultative chemolithoautotrophs that harbor low-affinity hydrogenases using H_2 plus O_2 for growth and energy production (Schwartz et al. 2013; Yang et al. 2019). This type of HOB is a vital primary producer using the Calvin-Benson-Bassham (CBB) cycle to assimilate the CO_2 into biomass, the same metabolic pathway as phototrophs, plants, and algae (Ortiz et al. 2021). The

scientific knowledge on HOB may become a blue route for reducing carbon footprint to combat climate warming independent of photosynthesis in soil (Pander et al. 2020; Ciani et al. 2021). In temperate soils (forests, grassland, wetland, farmland, uplands, and meadow) or extreme environments (cold and hot deserts) with atmospheric levels of H_2 (0.553 ppmv, ~ 400 pM in aqueous solution), most HOB adopt chemoheterotrophic growth mode. Representatives of this HOB type include *Mycobacterium smegmatis*, *Streptomyces spp.* and so on which consume H_2 through high affinity [NiFe]-hydrogenases (Constant et al. 2010; Greening et al. 2014a). Although H_2 is not sufficient to sustain the growth of HOB as the sole energy source in soils exposed to tropospheric H_2, it can support mixotrophic growth (Bay et al. 2021; Greening and Grinter 2022). The diversity and relative abundances of soil microbial taxa belonging to HOB play a crucial role in the functional biodiversity of soil (Fierer et al. 2012). Environmental factors such as H_2 concentration, O_2 concentration, nutrient availability, temperature, and moisture can be central factors driving the ecosystem-level distribution of HOB. Culture-dependent technologies and genome-resolved meta omics reveal the phylogeny and physiology of HOB and responses of soil bacterial communities to atmospheric- and elevated H_2.

Species interactions such as competition, commensalism, and cooperation count in understanding microbial community and refining biogeochemical processes (Großkopf and Soyer 2014; Palmer and Foster 2022). The research of hydrogenases lends itself as an approach to revealing relationships among the HOB as well as between this group and the bacteria in diverse niches (Schink and Schlegel 1978). The interactions between HOB and other bacteria provide insights into inferring the relationship between HOB activity and biogeochemical processes. Besides, knowledge about the ecological roles of HOB in ecosystems where H_2 concentrations are at or above tropospheric levels helps construct functionality-stable consortia for future biotechnological applications but is rarely summarized. Thus, this review also focuses on the mutually beneficial relationship of HOB-microbiota interactions and ecological roles of HOB in soil environments, i.e., agricultural soil, temperate soil, and desert. To summarize, this review highlights the diversity of HOB in physiology, distribution, isolation and identification techniques, hydrogenases, and metabolic strategy, giving an understanding of HOB from cells to ecosystems in soil.

2 Physiology, isolation, and identification

It is known that HOB is a physiological group with representatives in nearly all phyla (Yu 2018). It is necessary to introduce the physiological characteristics of the HOB population. Traditionally, isolation and enrichment of various HOB were detected through dilution-to-extinction and floating filter techniques. However, cultivation-dependent techniques have limitations in failing to grow most bacteria and quantifying the populations of HOB that possess hydrogenase activity. With molecular and meta omics techniques, more uncultured bacteria were identified as HOB in diverse soil niches.

2.1 Physiology

H_2 is a growth substrate for both aerobic and anaerobic H_2-oxidizing bacteria. Anaerobic H_2-oxidizing bacteria including sulfate reducers (e.g., *Desulfovibrio Vulgaris*), hydrogenotrophic denitrifiers (e.g., *Paracoccus denitrificans*), methanogens and acetogens (e.g., *Clostridium aceticum and Acetobaclerium woodii*) are not addressed in this article (Schink and Schlegel 1978; Aragno and Schlegel 1981; Wang et al. 2018). Aerobic HOB include both Gram-negative (*Pseudomonas*, *Alcaligenes*) and Gram-positive (*Corynebacterium*, *Mycobacterium*, *Nocardia*), mesophilic (*Acidobacteria*), thermophilic (*Hydrogenomonas Theophilus*), and psychrophiles (*Hymenobacter roseosalivarius*) (Bowien and Schlegel 1981; Madigan and Martinko 1997; Ortiz et al. 2021). HOB may have different metabolic processes and functions depending on environmental conditions.

2.2 Isolation

Many studies carried out enrichment and isolation of HOB from soil environments using dilution-to-extinction and floating filter techniques. First, a mineral medium with trace metal ions (especially Ni^{2+} and Fe^{2+}) was mainly used under a mixing gas composed of H_2, CO_2, and O_2 in the headspace (Ehsani et al. 2019). Besides, ammonia, urea, or even nitrogen gas can be supplied as nitrogen sources required

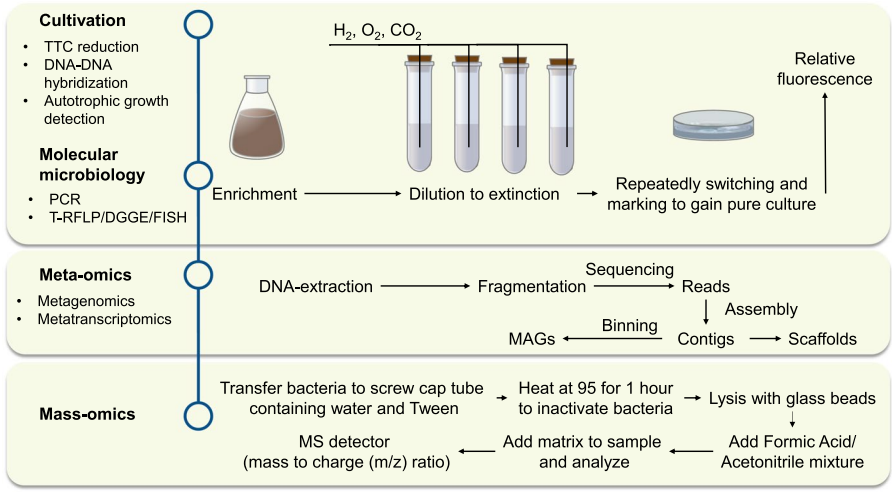


Fig.1 Summary of the development of identification techniques for HOB (Abiraami et al. 2020)

for biomass formation (Matassa et al. 2016; Pander et al. 2020; Hu et al. 2020). When the culture medium becomes muddy, the pure culture can be obtained by separating on solid medium in a glass jar containing the same gas mixture. Finally, pure culture will be obtained by repeatedly switching and marking (Madigan and Martinko 1997). Ehsani et al. (2019) established a feed mixture of H_2, O_2, and CO_2 (85:5:10) over five transfers to isolate *Hydrogenophaga electricum* as enriched autotrophic hydrogen-oxidizing microbiota. Pumphrey et al. (2011) enriched HOB colonies by serially diluting a soil and water mixture on a mineral medium containing 10 mM sodium bicarbonate. Then a pure culture of HOB was obtained after streaking the dilution mixture on R2A agar.

2.3 Identification

Selectively detecting and identifying HOB isolates have been developed to improve our knowledge of their ecology, diversity, and distribution (Fig. 1) (Lechner and Conrad 1997; Zhang et al. 2009; Pumphrey et al. 2011). Historically, HOB identification for metabolic and phenotypic assays can be separated into four types: (1) Cultivation-dependent techniques; (2) Molecular based techniques; (3) Gene sequencing-based molecular techniques; (4) Mass spectrometry. The principle of identification is simplicity, reliability, high specificity, high applicability, and low costs (Ehsani et al. 2019).

Characterization of HOB in the soil is conventionally studied by techniques based on the cultivation of soil microorganisms. Pure cultures of HOB were identified using H_2 oxidation ability, 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) reduction test, DNA-DNA hybridization, and autotrophic growth detection. The H_2 oxidation test of the isolated colony was carried out in a tube with a gas mixture of H_2, O_2, and CO_2, which is reliable for active HOB yet relatively time-consuming (Häring and Conrad 1994; Klüber et al. 1995). Another verification of HOB colonies is the TTC test, which irreversibly reduces water-soluble, uncolored TTC to the water-insoluble, red triphenylformazane. Although convenient and inexpensive, the TTC test may present false-positive results in the detection of some isolates unable to oxidize H_2 (Klüber et al. 1995). Another method of detecting HOB colonies was the DNA-DNA hybridization technique using DNA probes that possessed a hydrogenase gene (Klüber et al. 1995). A significant limitation is that DNA probes are too limited for detecting abundant HOB with various hydrogenases. Based on cultivation-dependent work, phylogenetically diverse and taxonomically widespread hydrogenase lineages have been identified (Greening et al. 2021). However, culture-based techniques have limitations in failing to grow most bacteria and quantifying the populations of HOB that possess hydrogenase activity (Klüber et al. 1995; Pumphrey et al. 2011).

Multiple molecular techniques, including polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), and terminal restriction fragment length polymorphism (T-RFLP) of soil microbial genes have

been applied to reveal microbial community profiles and drastically decreased the time of laboratory workflow. The most common culture-independent method to characterize community composition among habitats is the PCR technique based on the 16S rRNA gene (Lin et al. 2015). However, the phylogenetic diversity of the HOB makes it difficult to amplify 16S rRNA gene sequences of potential HOB using conventional PCR primers (Greening et al. 2015a). Besides, 16S rRNA gene analysis gives limited information on function. Probes and primers against the hydrogenase genes would be the preferred target for detecting HOB induced by H_2 (Islam et al. 2020). Developed quantitative real-time (qRT-PCR) techniques could further quantify genes and transcript copy numbers with absolute values instead of relative ones (Khdhiri et al. 2018). PCR analysis requires advanced knowledge of the characteristics of the particular HOB present to select the appropriate assay according to the testing application (Clark et al. 2013). Still, PCR is a powerful tool to explore variations of hydrogenase lineages and bacterial community members responsible for the H_2 biogeochemical cycle. FISH is one of the in-situ hybridization technologies that can perform on the extracted DNA or the rDNA products amplified by PCR-labeled oligonucleotide probes. FISH is suitable for identifying and quantifying microorganisms in specific taxonomic units (domains, genera, species, and subspecies) and detecting shifts in specific populations or species. Still, it is not suitable for describing community structure and overall diversity (Stein et al. 2005). Both T-RFLP and DGGE provide a pattern of "DNA fingerprints" bands unique for the particular DNA being analyzed in bacterial identifications. Compared to the more specific and targeted FISH technique, DNA fingerprints provide a broad survey of the entire community (Osborne et al. 2010). T-RFLP uses fluorescent-labeled primers in the PCR amplification process to label the PCR product by the end based on the principle of restriction fragment length polymorphisms (RFLPs). DNA fragments of different lengths are obtained by digesting a DNA sample with particular restriction endonucleases, which recognize very specific sequences of nucleotides in DNA (Osborne et al. 2006). These fragments are separated by gel or capillary electrophoresis with laser-induced fluorescence detection (Zhang et al. 2009). For example, Zhang et al. (2009) applied T-RFLP in H_2-treated soil and identified that the intensity differences of fragment peaks could be used to reflect population changes responsible for hydrogen metabolism in soil. Golding et al. (2012) carried out a T-RFLP analysis of barley soil samples and successfully distinguished two strains in H_2-treated soils. Stable-isotope probing can be combined with PCR to identify knallgas bacteria containing hydrogenase with ^{13}C-labeled DNA (Pumphrey et al. 2011).

Nowadays, meta omics emerge to reduce primer bias and shed light on the microbial diversity and the metabolic potential for HOB-mediated H_2 oxidation and CO_2 fixation (Picone et al. 2020). Illumina high-throughput sequencing (also known as next-generation sequencing, or NGS) is widely used to unveil the dynamics of the soil microbial diversity (Li et al. 2018). Genome-resolved metagenomic and meta-transcriptomics analyses have discovered many high-quality bins of HOB in the soil environmental samples, reflecting HOB community profiles in diverse soil ecosystems (Bay et al. 2021). Metabolomics analyses show metabolomes such as amino acids in cells when studying the mixotrophic growth of HOB and intermediary pathways to oxidize organic carbon. Despite high resolution for species determinations, inferences on HOB activity based on meta-omics are still challenging due to the diversity of HOB and the fragile linkage between hydrogenase gene abundance and functioning (Khdhiri et al. 2018; Picone et al. 2020).

Mass spectrometry is another powerful, quick, and accurate tool implemented for high-throughput dereplication and identification of bacterial isolates (Ehsani et al. 2019). Protein mass spectra displays mass-to-charge (m/z) ratios corresponding to ribosomal proteins, which is superior to nucleic acid probe and DNA strip methodologies. Although most isolates could be quickly and accurately identified to the species level, genetically similar HOB require sequencing of single or multiple targets to determinate species, adding to the difficulty in identification (Clark et al. 2013). Currently, applications of mass spectrometry have extended to combination with qRT-PCR amplicon sequencing to infer HOB activity and biochemical pathways involved in H_2 oxidation; combinations with metagenomics and isotope labeling to capture species identity, dependencies, and the nature of exchanged metabolites (Xu et al. 2019).

3 Diversity of HOB cells

All HOB contain one or more uptake hydrogenases which couple H_2 oxidation either to produce ATP through respiration or produce reducing power for carbon fixation (Vignais and Billoud 2007; Yu 2018). Although the hydrogenases perform the same reaction (Eq. 1) in the cell, each is linked to a different cellular process due to the diverse biochemical characteristics of hydrogenases.

$$H_2 + \frac{1}{2}O_2 \rightarrow H_2O \quad \Delta G'_0 = -237\ \text{kJ} \tag{1}$$

The diversity of hydrogenases induces the metabolic versatility of aerobic HOB. These microorganisms in soils can be categorized into two major groups with different hydrogenases and metabolic strategies, which correlate with different ecosystems. (a) Bacteria that use H_2 for chemolithoautotrophic growth, e.g., *Cupriavidus necator* (*Betaproteobacteria*), *Bradyrhizobium japonicum* (*Alphaproteobacteria*), *Methylacidiphilum spp.* (*Verrucomicrobia*). This process is typically mediated by low-affinity group 1d and 3d [NiFe]-hydrogenases. It is often associated with H_2-enriched environments such as root nodules in agricultural soil. (b) Bacteria that use H_2 for chemoheterotrophic growth and survival, e.g., *Mycobacterium smegmatis* (*Actinobacteriota*), *Streptomyces spp.* (*Actinobacteriota*), *Acidobacteria*. This process is typically mediated by high-affinity group 1 h and 2a [NiFe]-hydrogenases. This process is widespread in all soils worldwide, from drylands to forests to wetlands.

3.1 Hydrogenases

3.1.1 Classification

H_2 uptake by hydrogenases follows a biphasic first-order Michaelis–Menten kinetics using Lineweaver-Burke (Eq. 2) or Eadie-Hofstee plots (Eq. 3) (Maimaiti et al. 2007; Piché-Choquette et al. 2016).

$$\frac{1}{v} = \frac{K_m}{v_{max}}\frac{1}{[S]} + \frac{1}{v_{max}} \tag{2}$$

$$v = v_{max} - \frac{K_m}{[S]}v \tag{3}$$

where [S] represents H_2 substrate concentration (kmol m^{-3}), v refers to reaction rate (kmol m^{-3} s^{-1}), v_{max} is the maximum rate attained at very high H_2 concentrations, and K_m is the Michaelis constant (kmol m^{-3}). The reaction rate is one-half of the v_{max} at the substrate concentration equal to K_m. The accuracy of the Lineweaver-Burke Plot is greatly affected by the accuracy of data at low H_2 concentrations and the measured values of v appear in both coordinates thus the errors of v are superimposed in Eadie-Hofstee plots. H_2 oxidation activity suggests that hydrogenases can be classified as fast-acting, low-affinity (half-saturation constant [Km] > 1000 nM) and slow-acting, high-affinity (Km < 100 nM) hydrogenases (Häring and Conrad 1994). In temperate soils, *Actinobacteriota* (e.g. *Mycobacterium smegmatis*) are observed to oxidize trace H_2 (0.553 ppmv) given their high affinity (Km of ~ 50 nM) and low threshold (~ 50 pM) for H_2 (Greening et al. 2015b; Piché-Choquette et al. 2016). In contrast, *Cupriavidus necator* have a relatively high Km (Table 1) and are unable to utilize atmosphere H_2. However, they adopt a mixotrophic lifestyle utilizing organic substrates and atmospheric H_2 simultaneously in temperate soil (Schwartz et al. 2013). The Km of *Bradyrhizobium japonicum* was determined as 0.97 μM and can only grow efficiently on high levels of H_2 (Friedrich and Schwartz 1993; Van Soom et al. 1993).

According to the bi-metallic content of active sites, hydrogenases can be classified as three phylogenetically unrelated classes: the [NiFe]-, [FeFe]-, and [Fe]-hydrogenases (Vignais and Billoud 2007; Lubitz et al. 2014). [NiFe]- and [FeFe]-enzymes catalyze interconversion between H_2 and protons ($H_2 \leftrightarrow 2H^+ + 2e^-$) while the [Fe] only enzymes catalyze heterolytic cleavage of hydrogen ($H_2 \rightarrow H^+ + H^-$) (Parkin and Sargent 2012; Lu and Koo 2019). Among the three, [FeFe]-, [Fe]- hydrogenases are found in anaerobic H_2-evolving organisms and methanogenic archaea, representatively, which are out of the scope of this review. So far, [NiFe]-hydrogenases can be classified into four groups and 25 functionally distinct subgroups (groups 1a to 1 m, 2a to 2e, 3a to 3d, and 4a to 4f) based on phylogeny, predicted genetic organization, and biochemical characteristics (Vignais and Billoud 2007; Greening et al. 2016). Several subgroups of hydrogenases occur ubiquitously in aerobic HOB. The low-affinity subgroup 1d [NiFe]-hydrogenases supplying electrons to the respiration chain

were found in *B. japonicum* and *C. necator* (Greening et al. 2016). The high-affinity subgroup 1 h [NiFe]-hydrogenases (once classified as group 5) scavenge atmosphere H_2 when organic substrates are limited in abundant *Actinobacteriota*, *Acidobacteriota*, and *Chloroflexota* species (Xu et al. 2021). The subgroup 2a enzymes are also moderately or highly abundant in many soils, marine, and geothermal environments, and serve as a regulator of H_2 fluxes (Islam et al. 2020). The subgroup 2b [NiFe]-hydrogenases contain H_2 sensors which regulate the transcription of hydrogen operons (e.g., in *Rhodobacter capsulatus*). The subgroup 3b serves to interconvert electrons between H_2 and NADP in the cells (e.g., *Mycobacterium smegmatis*); the subgroup 3d bidirectional hydrogenases have to date been studied in detail in *Cupriavidus necator*, *Rhodococcus opacus* and cyanobacteria (Schwartz et al. 2013).

3.1.2 Biochemistry and genetic organization

Generally, [NiFe]-hydrogenases contain a large subunit and an electron-transferring small subunit accommodating three iron-sulfur (Fe-S) clusters (proximal, medial, and distal) (Schäfer et al. 2013). In the large subunit, Ni metallic group is the H_2-binding site and is anchored via four thiol groups of the cysteine residues, two of which bridge ligands to the Fe atom. The Fe coordination is completed by two cyanides and one carbon monoxide ligand (Ludwig et al. 2009; Greening et al. 2015b). The chemical structures of the active site are conserved (Greening et al. 2016). However, the configuration and ligands of three small Fe-S clusters differ between subgroups, in which Cys, Asp, Glu, Asn, and His can be nonstandard ligands. Electrons from H_2 relay in the direction of the catalytic center, the proximal cluster of the small subunit, medial cluster, distal cluster, and protein surface (Lu and Koo 2019). The Fe-S clusters function as a ''conductive wire''. The proximal [4Fe-4S] cluster can exchange electrons directly with the active site (Lenz et al. 2010). A medial [3Fe-4S] cluster protects the enzyme from inactivation by oxygen (Fritsch et al. 2011). [4Fe-4S] cluster is one of the most common distal clusters of the hydrogenases which is coordinated by three cysteines and one histidine residue. This histidine appears to mediate the electronic exchange between the hydrogenase and its corresponding redox partner (Fritsch et al. 2011).

The differences in the biochemical characteristics of hydrogenases occur within various taxonomic groups of bacteria, enabling HOB to support life across a wide range of ecological niches (Table 1). There are four different [NiFe]-hydrogenases groups in the representative *Cupriavidus necator* (once classified as *Ralstonia eutropha* and *Alcaligenes eutrophus*) on the pHG1 megaplasmid: (1) membrane-bound respiratory enzymes (MBH; subgroup 1d); (2) soluble enzymes dependent on NADH/NADPH or $NAD^+/NADP^+$ as cofactors (SH; subgroup 3d); (3) cytoplasmic H_2-sensing proteins (RH, subgroup 2b); and (4) an Actinobacteriotal hydrogenase (AH, subgroup 1 h) (Schäfer et al. 2013). The heterotrimeric MBH consists of a small and a large subunit encoded by HoxK and HoxG, as well as a third subunit HoxZ with two heme molecules related to cytochrome b. This HoxZ protein anchors MBH to the cytoplasm membrane via hydrophobic C-terminal transmembrane helical segment (Fritsch et al. 2011). The heterodimeric AH is synthesized at low levels which transfers the electrons from H_2 to an unknown electron acceptor (Palmer and Berks 2012; Schwartz et al. 2013). AH has a low oxidation activity and is unable to sustain autotrophic growth when the remaining hydrogenases are missing (Schäfer et al. 2013). RH shares significant sequence identity with the MBH (Lenz and Friedrich 1998), in which HoxB and HoxC comprise a $\alpha_2\beta_2$ dispensable tetramer (Bernhard et al. 2001). A specific peptide at the C terminus of the small subunit HoxB mediates the interaction of the histidine kinase HoxJ with the tetramer (Schwartz et al. 2013). HoxJ specifically senses H_2 as part of a regulatory cascade, which controls the response regulator HoxA (Buhrke et al. 2004; Greening and Cook 2014). The HoxA, in its non-phosphorylated form, activates the transcription of MBH and SH with the availability of hydrogen and organic carbon (Zimmer et al. 1995). The heterohexameric SH forms an electron transport chain from H_2 to NAD^+. The active site (HoxH) transfers electrons to an FMN-a cofactor, then to the [Fe-S] clusters of the small subunit (HoxU), then to another FMN-b cofactor, and finally to NAD^+ (Burgdorf et al. 2005a). Two subunits of HoxI forming a complex with the diaphorase moiety have a regulatory function and may be used to bind $NADP^+$ as an alternative electron acceptor (Burgdorf et al. 2005a).

Table 1 Biochemical characteristics of purified hydrogenases in model microorganisms

Organism	Hydrogenase	Group	Function	Small subunit			O_2 tolerance	Km(H_2)[a] (μM)	References
				proximal	medial	distal			
Cupriavidus necator	MBH	1d	Energy conserving	6Cys[4Fe3S]	3Cys[3Fe4S]	3Cys1His[4Fe4S]	Tolerant	6.1	(Schäfer et al. 2013)
	RH	2b	H_2 sensor	[4Fe4S]	[4Fe4S]	[4Fe4S]	Tolerant	25	
	AH	1h	Unable to uptake atmospheric H_2	3Cys1Asp[4Fe4S]	4Cys[4Fe4S]	3Cys1His[4Fe4S]	Tolerant	4.9 ± 1.2 (15 °C); 3.6 ± 0.5 (30 °C)	
	SH	3d	Energy conserving	[4Fe4S]	[4Fe4S]	[2Fe2S]	Reversibly inhibited	11	
Bradyrhizobium japonicum	Hup	1d	Recycle H_2 produced by nitrogenase	[4Fe3S]	[3Fe4S]	[4Fe4S]	Tolerant	0.97	(Friedrich and Schwartz 1993; Van Soom et al. 1993)
Mycobacterium smegmatis	Hhy	1 h	Membrane-bound	3Cys1Asp[4Fe4S]	4Cys[4Fe4S]	3Cys1His[4Fe4S_1]	Completely insensitive	0.05	(Greening et al. 2014a; Søndergaard et al. 2016)
	Huc	2a	Soluble	[3Fe4S]	[4Fe4S]	[4Fe4S]	Tolerant	0.18	
	Hyh	3b	Redox sink	[4Fe4S]	[4Fe4S]	[4Fe4S]	Tolerant	High affinity	
Hymenobacter roseosalivarius	Hyl	1 l	Uptake atmospheric H_2	4Cys[4Fe4S]	4Cys[4Fe4S]	3Cys1His[4Fe4S_1]	Tolerant	High affinity	(Ortiz et al. 2021)

[a]The kinetic parameters were determined on isolated enzymes using different methods and are therefore only comparable to a limited extent

In agricultural soil, *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* are typical Hup + rhizobia strains that recycle H_2 produced by nitrogenase within the legume nodule, thereby improving symbiotic nitrogen fixation (Van Soom et al. 1993; Baginsky et al. 2005). In contrast to *B. japonicum*, *R. leguminosarum* strains show similar conserved sequences and gene organization in uptake hydrogenases (Hup) but are unable to fix CO_2 autotrophically (Friedrich and Schwartz 1993). The hupS and hupL genes encode the structural subunits. Differences exist in the regulation of specific genes. *B. japonicum* expresses hup genes in symbiosis and microaerobic free-living cells, while *R. leguminosarum* expresses only in symbiotic conditions with peas (Brito et al. 2002). The regulatory circuits that control *B. japonicum* hydrogenase gene expression include HupUV sensing environmental factors such as O_2, H_2, and Ni; the response regulator HoxA (free-living condition); and the repressor HoxX. Instead of HoxA, symbiotic transcription of the hupSL promoter is activated by NifA proteins in *R. leguminosarum*, which is the central regulator for nitrogen fixation (Brito et al. 1997). Besides, the hyp accessory genes are expressed under the control of an Fnr-like transcriptional activator. This unique regulation pattern of *R. leguminosarum* appears to result from an adaptive evolution toward temporally and spatially coregulation of the hydrogenase and nitrogenase systems in pea nodules (Ruiz-Argüeso et al. 2001).

In temperate soils, saprophyte *Mycobacterium smegmatis* was identified ubiquitously containing three [NiFe] hydrogenases, designated Huc (subgroup 2a), Hhy (subgroup 1 h), and Hyh (subgroup 3b). Both Huc and Hhy are oxygen-tolerant, and oxidize H_2 to subatmospheric concentrations, while Hyh is expressed to recycle reduced equivalents by evolving H_2 (Berney et al. 2014; Islam et al. 2019b, 2020; Cordero et al. 2019). In Antarctic soil, representative

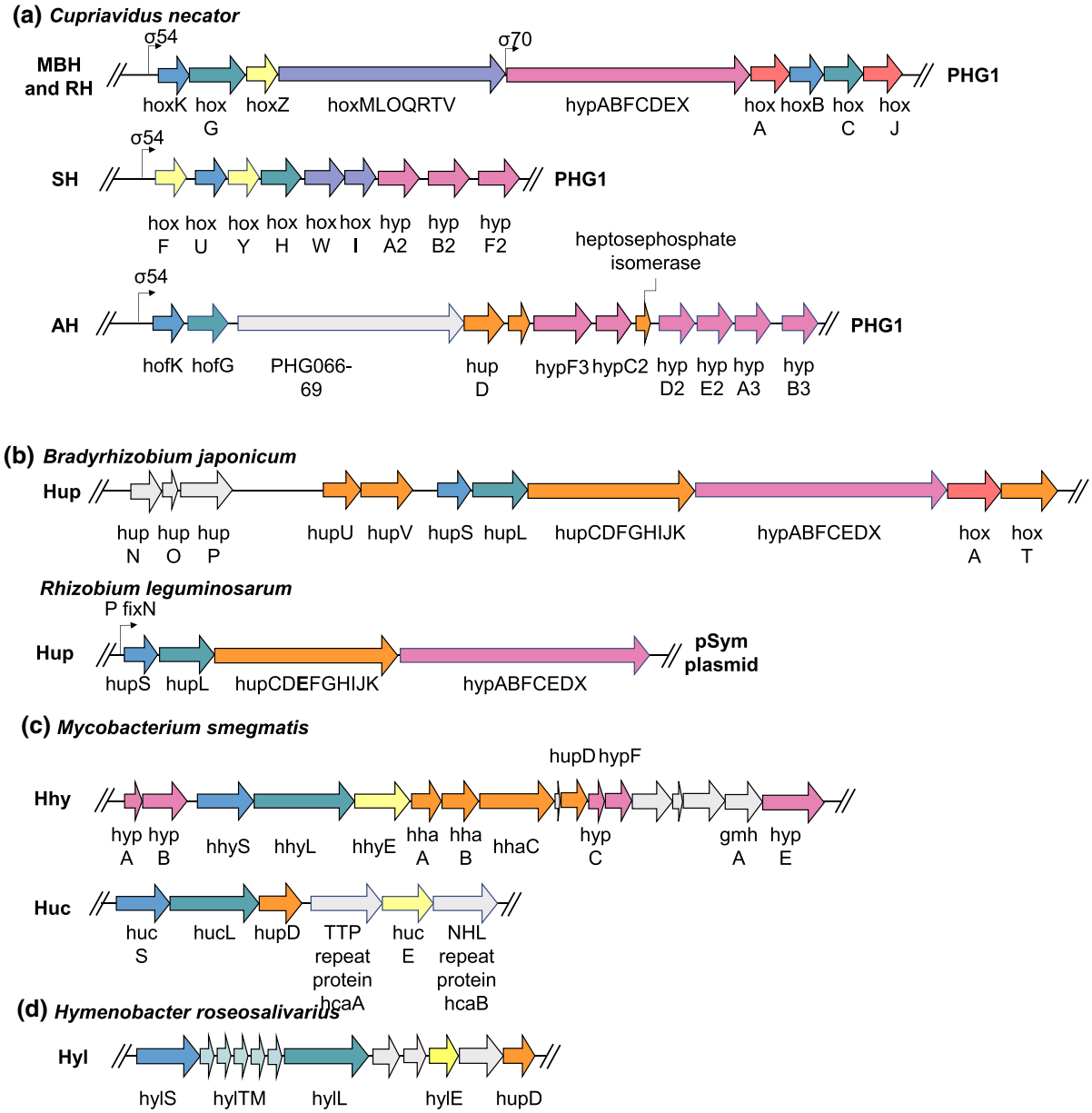


Fig. 2 Comparison of representative genetic organization of [NiFe]-hydrogenase gene cluster derived from **a** *Cupriavidus necator* (group 1d, 3d, 1h, 2b) (Panich et al. 2021); **b** *Rhizobium leguminosarum and Bradyrhizobium japonicum (*group *1d)* (Ruiz-Argüeso et al. 2001); **c** *Mycobacterium smegmatis* (group 1h, 2a) (Greening et al. 2016; Islam et al. 2019b); (d) the Antarctic bacterium *Hymenobacter roseosalivarius* (group 1l) (Ortiz et al. 2021). Encoded proteins are colored as follows: yellow = Electron-relaying or Rieske-type protein; green = Large subunit; blue = Small subunit; grey = Hypothetical proteins; orange = Hydrogenase maturation endopeptidase (HupD); light blue = Single-pass transmembrane proteins (HylTM); pink = Hydrogenase assembly (HypABCDEFX); purple = accessory function proteins; red = regulatory module

HOB *Hymenobacter roseosalivarius* harbors a high-affinity Hyl hydrogenase (subgroup 1 l) that consists of the structural subunits HylS and HylL (Ortiz et al. 2021). The gene organization is displayed in Fig. 2.

In almost all HOB species, the hydrogenases genes are clustered in functional groups: structural genes encoding the subunits and a cytochrome-like (e.g., HoxZ in *C. necator*) or Rieske-type protein (HhyE

and HucE in *M. smegmatis*) mediating electron transfer between the catalytic subunits of the hydrogenases; genes for accessory proteins designated as hyp (Friedrich and Schwartz 1993; Islam et al. 2019b). The complete set of Hyp proteins is mandatory for all [NiFe]-hydrogenases and is responsible for the maturation of hydrogenases (Lin et al. 2022). The HypB has GTPase activity and is involved in nickel ions binding (Fu et al. 1995). The HypW protein (e.g., in *C. necator*) or HupD protein (e.g., in *M. smegmatis, B. japonicum,* and *H. roseosalivarius*) resembles the maturation endopeptidases which remove the C-terminal extension of the large subunit precursor (Schäfer et al. 2013). The mechanisms governing the expression of hydrogenase genes vary depending on the physiological context. In obligate fermenters, for instance, the expression of hydrogenase genes is typically constitutive. In *C. necator* and *B. japonicum*, hydrogenase expression is regulated by response-regulator-type transcriptional activators encoded by the hoxA gene, which requires σ54-dependent RNA polymerases to induce hydrogenase structural genes (Friedrich and Schwartz 1993; Durmowicz and Maier 1997).

Structural differences in hydrogenases may also contribute to differences in O_2 tolerance. Hydrogen metabolism predominantly occurs in anoxic environments mediated by O_2-sensitive [NiFe]-hydrogenases (referred to as the standard hydrogenases) (Ludwig et al. 2009; Lu and Koo 2019). The standard enzymes were inactivated to a resting Ni–A or Ni–B state due to O_2 reduction at the [NiFe] active site. The process can be described as electrons transferring from Fe-S relay centers to the active center in the reverse direction of H_2 oxidation. Four electrons are required to form the Ni–B state: one provided by the [NiFe] center itself from Ni(II) to Ni(III); the other three from proximal, medial, and distal clusters, respectively. The active site in the Ni–B state can be rapidly re-activated once obtained one electron through reversed electron transfer. In standard hydrogenases, the third electron from the medial to the proximal cluster faces an 'uphill' hurdle of reduction potential thereby posing a Ni-A Unready state accumulates. Ni-A is kinetically unfavorable to return to the active state thus hydrogenases were observed inactivated (Parkin and Sargent 2012). It was later found that MBH in *Ralstonia eutropha* H16 can perform H_2-oxidation in a high oxygen atmosphere (Burgdorf et al. 2005a; Shomura et al. 2011; Yu 2018). For MBH (group 1d hydrogenase), oxygen tolerance is mediated by a unique proximal 6Cys[4Fe3S] cluster ligated by six cysteines. The cluster is stable in three redox states ($1Fe^{2+}$:$3Fe^{3+}$/$2Fe^{2+}$:$2Fe^{3+}$/$3Fe^{2+}$:$1Fe^{3+}$), and thus it can donate two electrons from reduced $[4Fe3S]^{3+}$ to superoxidized $[4Fe3S]^{5+}$ which means the electron is not needed from the distal cluster to generate Ni–B state (Goris et al. 2011; Parkin and Sargent 2012; Frielingsdorf et al. 2014). Besides, overall reduction potentials of the [Fe-S] clusters in MBH become 'flattening', which makes it thermodynamically easier to generate a Ni–B state upon O_2 attack (Parkin and Sargent 2012). The well-reported proximal cluster is exclusive to group 1d enzymes. Some other hydrogenases independently developed mechanisms to prevent or reverse the formation of O_2-inactive states (Fritsch et al. 2011). Similar to the mechanism of the MBH, reverse electron transfer to the [NiFe] active site of the SH is assumed to mediate the efficient neutralization of O_2 attacking the active site (Fritsch et al. 2013). The SH (subgroup 3d) is inactive in the oxidized state but can also be reactivated by various reducing agents such as NADH and NADPH (Burgdorf et al. 2005b). O_2 tolerance of RH (subgroup 2b) from *C. necator* arises from the bulky residues isoleucine and phenylalanine, providing a narrow gas channel restricting access of O_2 to the active site (Buhrke et al. 2005; Parkin and Sargent 2012). AH (subgroup 1 h) from *C. necator* maintained full activity even in the presence of 70% O_2 due to a selective filter in gas channels that excludes small molecules such as O_2 and CO from the active site (Schäfer et al. 2013).

3.2 Metabolic versatility

3.2.1 Bacteria that use hydrogen for chemolithoautotrophic growth

Aerobic H_2-oxidizing bacteria that use H_2 for chemolithoautotrophic growth are also called knallgas bacteria. The process of H_2 oxidation is typically mediated by low-affinity group 1d and 3d [NiFe]-hydrogenases (releasing electrons for aerobic respiration and carbon fixation respectively) in conjunction with ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCO). They are mostly not able to metabolize atmospheric H_2 but can thrive organotrophically. However, it has

been found that some autotrophic HOB (e.g., *Methylacidiphilum fumariolicum SolV* and *Pseudonocardia spp.*) with high-affinity 1 h [NiFe]-hydrogenases are exceptions that can fix CO_2 into biomass using atmospheric trace gases. These special organisms encode complete aerobic heterotrophic and autotrophic carbon acquisition pathways (Grostern and Alvarez-Cohen 2013; Schmitz et al. 2020). During the transition from exponential to stationary phase, i.e., the depletion of carbon sources, atmospheric H_2 scavenging is triggered in *Methylacidiphilum fumariolicum SolV* to support persistence (Greening et al. 2015a).

C. necator (Gram-negative) displays a representative mode of facultative H_2 chemolithoautotrophs (Fig. 3a). The bacterium balances heterotrophic and lithotrophic growth modes by responding to carbon sources and energy states (Greening and Cook 2014). *C. necator* can utilize organic substrates or grow mixotrophically utilizing organic substrates combined with H_2 as energy sources when H_2 is below the minimum concentration that they can oxidize (Pumphrey et al. 2011). During exponential growth on carbon substrates of succinate, pyruvate, or acetate, the synthesis of key enzymes in the CBB cycle and hydrogenases was found to be derepressed (Schwartz et al. 2009). Instead, these enzymes were expressed under conditions of limited availability of energy (e.g., grow on glycerol, fructose, gluconate, or citrate) (Friedrich et al. 1981; Schwartz et al. 2009). Furthermore, in the absence of organic substrates, they live on H_2 and CO_2 as their sole sources of energy and carbon (Schwartz et al. 2013). As discussed above in 3.1.2, MBH (group 1d) and SH (group 3d) are energy-conserving hydrogenases that link to the respiratory chain via a b-type cytochrome and the reduction of NAD^+, respectively. MBH attached to the periplasmic side of the cytoplasmic membrane is found in many proteobacteria coupling the electron flow to the quinone pool of the respiratory chain. Electrons generated by H_2 oxidation of MBH with the most negative E_0' ($2H^+/H_2$, −0.414 V) are transferred to ubiquinone, cytochrome bc1 complex, cytochrome c in turn, and finally, to cytochrome bc3 oxidase utilizing O_2 as electron acceptor (Greening and Cook 2014). The terminal oxidase complex extrudes H^+ to the membrane's outer surface, resulting in the accumulation of OH- inside the membrane and the generation of a proton motive force (Madigan and Martinko 1997). Some of the potential energy is used in a proton gradient to pump hydrogen through ATP synthase, which generates ATP for cells and spores to persist in the absence of organic carbon (Greening et al. 2015b). During the lithotrophic growth mode, *C. necator* incorporates CO_2 into cell fraction through the CBB cycle with RuBisCO (Ray et al. 2022). The reaction can be described as (Eq. 4):

$$21.36H_2 + 6.21O_2 + 4.09CO_2 + 0.76NH_3 \rightarrow 6C_{4.09}H_{7.13}O_{1.89}N_{0.76} + 19.70H_2O \quad (4)$$

The yields of biomass are 4.6 g dry weight/mol H_2, higher than that of *Kyrpidia spormannii FAVT5* (4.3 g dry weight/mol H_2) and *Methylacidiphilum fumariolicum SolV* (3.4 g dry weight/mol H_2) in geothermal soils (Hogendoorn et al. 2020). During periods of nutrient limitation or low-oxygen concentration, the growth of the cells is inhibited and storage material poly-β hydroxybutyric acid (PHB) is synthesized (Matassa et al. 2015). The stoichiometric relationship of PHB accumulation agrees with Eq. 5 (H. G. Schlegel et al. 1961; Jannasch and Mottl 1985).

$$25H_2 + 8O_2 + 4CO_2 \rightarrow C_4H_6O_2 + 22H_2O \quad (5)$$

Bradyrhizobium japonicum is another facultative chemolithoautotroph in the group of diazotrophs synthesizing group 1d [NiFe] hydrogenase to recycle H_2 produced by nitrogenase reaction (Maier and Triplett 1996). Under free-living conditions outside the root nodule, *B. japonicum* expresses RubisCO and hydrogenase activity simultaneously and grows on H_2 and CO_2 as sole sources of energy and carbon (Purohit et al. 1982). Under symbiotic conditions, however, hydrogenase expression is closely tied to nif control in nitrogen fixation (Durmowicz and Maier 1997). This dual strategy is important for inhabitants of the rhizosphere.

3.2.2 *Bacteria that use hydrogen for chemoheterotrophic persistence*

Chemoheterotrophic HOB using H_2 for growth and survival are typically mediated by high-affinity group 1h and 2a [NiFe]-hydrogenases. Hydrogenase expression and activity increase in carbon-limited cells, suggesting that scavenging of trace H_2 helps to sustain dormancy. Soil organisms harboring high-affinity

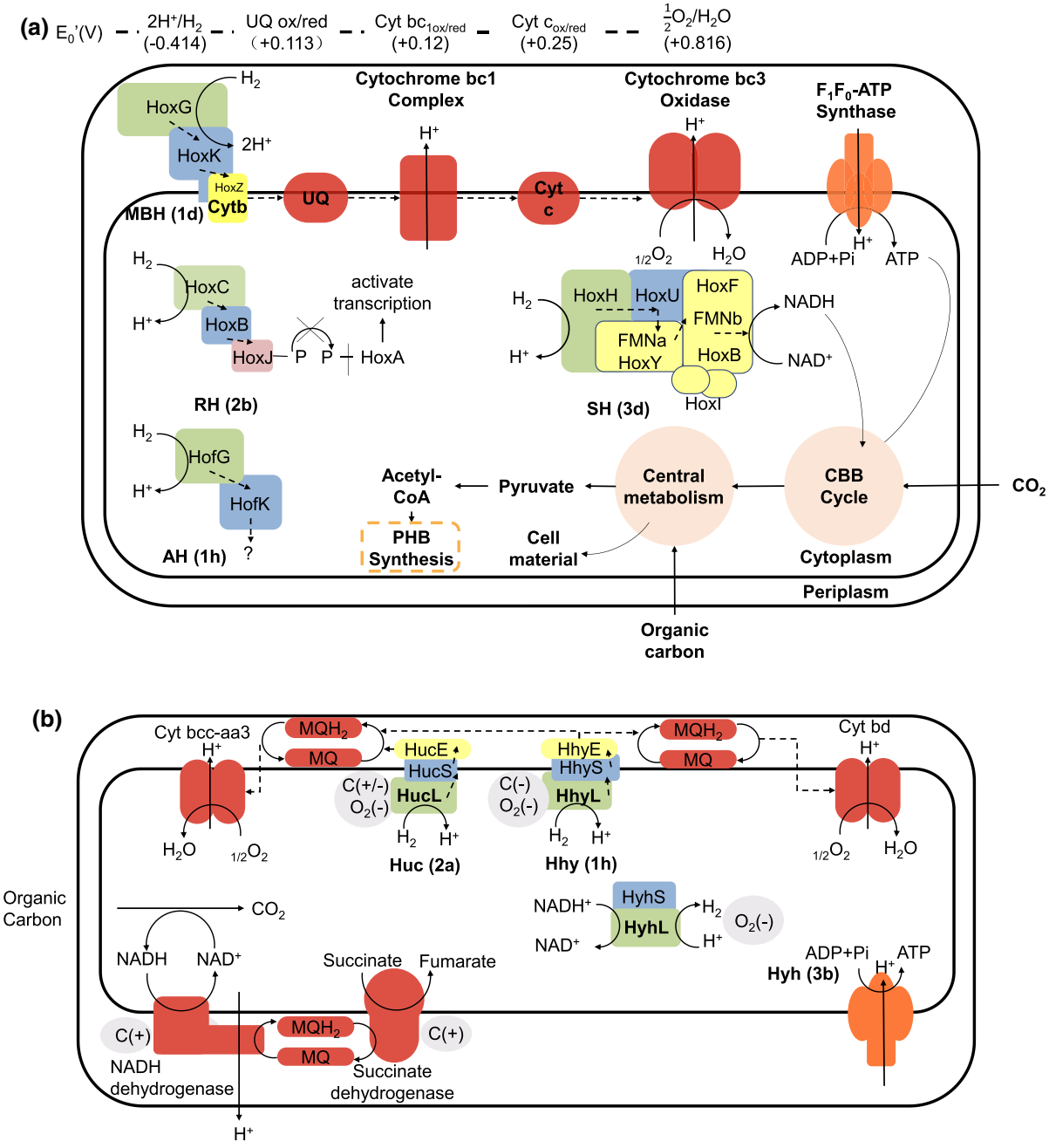


Fig. 3 Metabolic processes in **a** Cupriavidus necator; **b** Mycobacterium smegmatis. Thick dashed line: a vertical tower of electron transfer during H_2 oxidation; Thin dashed arrow: direction of electron flow; (+) and (−) represent cell state determining when the enzyme is most synthesized: C(+) for growth state; C(−) for starvation; O_2(−) for hypoxia; C(±) for transition. Encoded proteins are colored as follows: Red: enzymes involved in the electron-transport chain; Green: the large subunit of the hydrogenase; Blue: the small subunit of the hydrogenase; Yellow: electron transfer clusters of the hydrogenase; Pink: histidine kinase; Orange: ATP synthase (Greening and Cook 2014; Frielingsdorf et al. 2014; Yu 2018; Bay et al. 2021; Greening et al. 2021; Greening and Grinter 2022)

hydrogenases may be especially competitive, given that they harness a highly dependable fuel source in otherwise unstable environments. Group 2a hydrogenases are upregulated during exponential growth and for group 1h hydrogenases, it is linked with persistence.

As one of the chemoheterotrophic obligate aerobes, *M. smegmatis* (Gram-positive) is widely reported for its flexibility in the metabolic process (Fig. 3b). *M. smegmatis* lacks a gene cluster for carbon fixation that renders it incapable of autotrophic growth but it is capable of mixotrophic growth combining atmospheric H_2 and organic carbon oxidation (Greening et al. 2014b). In the carbon-limitation environment, respiratory chain components and the F1Fo-ATP synthase of the cells are down-regulated and the cells adopt a reduced growth rate (Berney and Cook 2010). This was paralleled by the up-regulation of hydrogenases Hhy (group 1h) to maintain the flow of reducing equivalents to the electron transport chain during dormancy (Berney and Cook 2010). By contrast, Huc (group 2a) is most synthesized during the transition from growth to persistence (Cordero et al. 2019). Both Huc and Hhy transfer electrons into the respiratory chain via the menaquinone pool for energy conservation (Piché-Choquette et al. 2016; Cordero et al. 2019; Xu et al. 2021). The difference in electron transferring is that Huc exclusively donates electrons to the proton-pumping cytochrome bcc-aa3 super complex while Hhy also provides electrons to the cytochrome bd oxidase complex (Cordero et al. 2019). All three hydrogenases were found to be upregulated under hypoxia combating reductive stress. Hyh recycles reductants by fermentatively evolving H_2 combined with NADH oxidation regulated by DosR during hypoxia (Berney et al. 2014). Taken together, hydrogen metabolism enables *M. smegmatis* to rapidly meet its energy needs in response to O_2 and organic carbon availability and provides a competitive advantage in low oxygen and carbon environments (Xu et al. 2021).

Constant' lab isolated the first high-affinity *Streptomyces sp. PCB7* (*Actinobacteriota*) from soil. This strain is capable of utilizing atmospheric H_2 down to a threshold concentration of 0.1 ppmv utilizing high-affinity group 1h hydrogenases (Constant et al. 2008, 2011a). Later research found that high-affinity H_2 uptake activity is widespread among the *Streptomyces spp.* isolated from temperate forests and agricultural soils (Constant et al. 2010). Hydrogenase activity is proportional to the abundance of HOB mainly composed of Streptomyces spp. in soil (Constant et al. 2011b). *Streptomyces spp.* exhibiting high affinity 1 h enzyme are primarily expressed in spores to support a seed bank under a mixotrophic survival energy mode (Liot and Constant 2016). And this H_2 oxidation process is responsible for the most important sink in the atmospheric H_2 budget (Constant et al. 2010).

3.3 Responses of HOB to ecological factors

The microbial community continues to change over time depending on rapidly and frequently varying environmental conditions (Wang et al. 2020a). HOB regulate H_2 metabolism via the transcription of hydrogenases to adapt to physicochemical variables (Greening and Cook 2014). Experimental evidence supporting ecological factors that drive the H_2 oxidation activity, distribution of hydrogenases, and community structure in diverse soil niches are still scarce. Recent studies found that environmental variables such as electron donors and acceptors, nutrient pools, moisture, and temperature play important roles in structuring HOB taxa, microbial fitness, and interactions. Here, Fig. 4 summarizes the ecological factors influencing H_2 metabolism in soil niches.

3.3.1 Electron donors and acceptors

H_2 and O_2 are electron donors and acceptors in the H_2 metabolism of HOB. H_2 concentration varies by magnitude spatially and temporally from 400 pM on surfaces of aerobic soils (Constant et al. 2009) to 40 mM in anaerobic gastrointestinal tracts (Olson and Maier 2002). The impacts of H_2 content on hydrogenase expression and microbial communities were investigated in several studies. At the gene expression level, hydrogenase formation may be H2-dependent or independent. In some HOB such as *C. necator* and the symbiotic N_2-fixer *R. leguminosarum*, hydrogenase expression is not controlled by H_2 availability (Schwartz et al. 2009, 2013). In contrast, hydrogenase formation of *Alcaligenes Hydrogenophilus*, *B. japonicum*, and *R. capsulatus*, requires the presence of H_2 and is induced by H_2 during growth (Schlegel and Meyer 1985). It was reported that the hup promoter

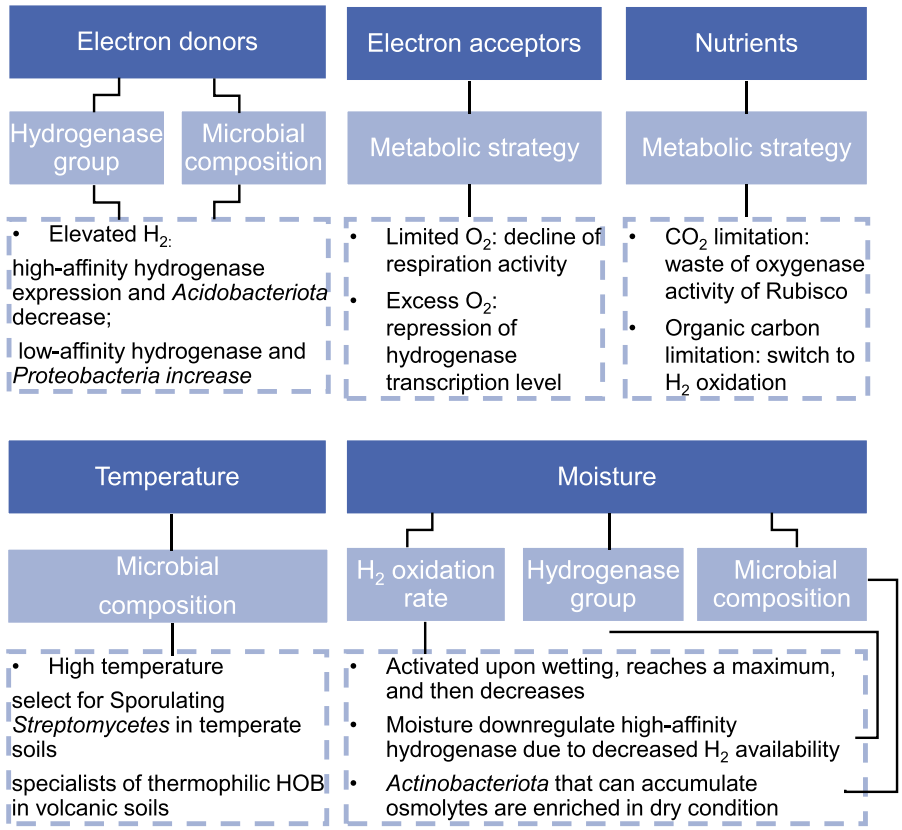


Fig. 4 Ecological factors influencing H_2 metabolism in soil niches

activity in *B. japonicum* was activated in response to H_2 partial pressure over a range of 0.05–3% (Friedrich and Schwartz 1993). H_2 availability regulates the synthesis levels of different groups of [NiFe]-hydrogenases (Khdhiri et al. 2018). After 10–15 d of exposure to elevated H_2 (525 or 10,000 ppmv), a significant decrease of group 1 h [NiFe]-hydrogenase expression was observed using qPCR and qRT-PCR techniques in the farmland, poplar, and larch soil microcosms. At the same time, the expression of genes encoding low-affinity hydrogenases was upregulated (Piché-Choquette et al. 2016, 2017). At the taxonomic level, H_2 drives changes in the structure of microbial communities with uneven responses among different taxonomic groups in soil ecosystems (Zhang et al. 2009). The apparent affinity of HOB (i.e., Km) towards H_2 increased after two weeks of exposure to elevated H_2 (10,000 ppmv), indicating the enrichment of low-affinity HOB (Wang et al. 2020a). High pH_2 would select for the low-affinity HOB, e.g., *Proteobacteria* harboring group 1d [NiFe]-hydrogenases combined with the detrimental effect on the high-affinity HOB e.g., *Acidobacteriota* (Smith-Downey et al. 2008; Khdhiri et al. 2018). In contrast, both mediate and low H_2 concentrations select for high-affinity group 1h and 2a hydrogenases encoded by sporulating or aerial hyphae forming *Streptomycete* and persistent *Mycobacteria* (Greening et al. 2015b). pH_2 also appears to influence the metabolic process of HOB. Under H_2 limitation, high-affinity hydrogenases enable the respiration activity to keep at a high level (Yu 2018). However, more H_2 was used for energy supply, unfavorable for CO_2 fixation (Yu et al. 2013).

Oxygen content is another key factor controlling the transcriptional level and activity of respiratory hydrogenases (Greening et al. 2016). At the transcription level, it was found that O_2 partial pressures affected the activity of the hup promoter in *B. japonicum*. At O_2 partial pressures of 0.1–3%, hydrogenases transcription directed by the hup promoter maintained at high levels in the presence of H_2 but repressed sharply above 3% (Friedrich and Schwartz 1993). At the activity level, the H_2 uptake rate reached a maximum level of 200 mmol H_2 L^{-1} h^{-1} under oxygen

limitation. However, the respiration activity declined quickly with time. In general, chemolithotrophic HOB require a H_2/O_2 molar ratio of about 3 to maintain a balance between energy supply by respiration and CO_2 fixation (Yu 2018).

3.3.2 Nutrients

The essential nutrients for HOB cell growth contain carbon sources, bioavailable nitrogen, sulfur, sodium, phosphorus, potassium, manganese, copper, zinc, boron, aluminum, iron, and silicon (Yu 2018; Bay et al. 2021). Most soil ecosystems are characterized by many recalcitrant organic polymers and competition for easily degradable organic sources. Organic carbon has become the main factor limiting microbial growth with spatiotemporal variability (Bay et al. 2021). In nature, most HOB shift between chemoorganotrophic and chemolithoautotrophic lifestyles depending on nutrient availability in their habitats. The flexibility to oxidize H_2 as a backup metabolism to organic compounds oxidation enables them a selective advantage over organotrophs. Previous evidence has shown that bacterial cultures from *Actinobacteriota*, *Proteobacteria*, *Acidobacteriota*, and *Chloroflexota* phyla switch from growth on organic carbon to persistence on H_2 gases responding to carbon starvation (Greening et al. 2021). Synthesis of the CBB cycle and H_2 oxidation by HOB is repressed when readily organic compounds are available (Madigan and Martinko 1997). Under CO_2 limitation (< 1% mol CO_2), large amounts of hydrogen were utilized for respiratory and the oxygenase activity of Rubisco was wasted, causing ribose-1,5-bisphosphate futilely cleaved into 2-phosphoglycolate (Yu 2018).

3.3.3 Temperature and moisture

Drivers of soil HOB composition also include temperature and moisture. The optimal growth temperature of HOB varies according to the thermostability of hydrogenases. It has been found that hydrogenase activity changes with temperature (Chowdhury and Conrad 2010). In H_2-exposure experiments of German forests and garden soils, H_2 soil uptake optimal temperatures were observed to be 35–40 °C for high-affinity activity and 50–60 °C for low-affinity activity between 5 and 60 °C (Schuler and Conrad 1991). In a temperate ecosystem, soil H_2 uptake was positively correlated with soil temperature (Meredith et al. 2017). The hydrogenases in certain mesophilic *Actinobacteriota*, especially sporulating *Streptomycetes* have a thermophilic origin, ensuring energy generation even under deleterious conditions (Greening et al. 2015b). At high temperatures, temperature-sensitive hydrogenases are killed and the half-life of H_2 oxidation activity becomes significantly shorter. In volcanic soils, distinct environmental niches select for specialists of thermophilic HOB, e.g., genus *Methylacidimicrobium* which could grow optimally at 50 °C in a pH range of 3–5 (Picone et al. 2021).

The impacts of moisture on H_2 oxidation rate, hydrogenase groups, and microbial communities are summarized. Plant root nodules and fermentation reactions producing H_2 usually take place in waterlogged niches (Khdhiri et al. 2018). However, excessive moisture in the soil caused H_2 and O_2 diffusion limitation, leading to the variation of community composition from aerobic bacteria to facultative anaerobes, and finally to strict anaerobes (Li et al. 2018; Hartman and Tringe 2019). In contrast, drought stress induced osmotic stress and reduced nutrient diffusion, especially in desert soil (Smith-Downey et al. 2008; Hartman and Tringe 2019). Besides, drought leads to the starvation of microbes that fail to move and find uptake resources (Schimel 2018). At the H_2 consumption rate level, Edao and Iwai (2020) found that the soil was deactivated under the condition of dry air feeding and the conversion rate of H_2 gradually decreased with time. Recovered H_2 oxidation activity was observed after the soil was soaked in water. Another study found that the rate constants were generally lower at 60% than at 30% water holding capacity due to H_2 diffusion limitation (Gödde et al. 2000). These studies have implied that the H_2 oxidation activity in soil is rapidly activated upon wetting, reaches a maximum, and then decreases. At the functional group level, soil moisture induces variation in the expression of hydrogenases (Wang et al. 2020a). *Actinobacteriota*-associated high-affinity [NiFe]-hydrogenase (subgroup 1 l) decreased following hydration (Jordaan et al. 2020). Decreased H_2 availability account for the negative correlation between moisture and abundances of high-affinity hydrogenase in water-saturated soil (Wang et al., 2020a, b). At the community composition level, HOB members adapt to moisture conditions by combining the respiration of exogenous organic carbon with H_2

oxidation in dry conditions. Spore-forming *Actinobacteriota* has competitive advantages in dry conditions as they accumulate osmolytes inside cells to lower internal solute potential and avoid losing water to soils. In addition, as gram-positive bacteria, their cell wall layer is thought to improve their ability to resist desiccation. After hydration, organic carbon becomes more bioavailable and HOB rapidly replenishes organic carbon amid intense competition, which induces downregulation of mixotrophic metabolism. In the rhizosphere of agricultural soil, moisture indirectly increases the number of HOB as it is favorable for legume nodules that produce H_2 during nitrogen fixation (Li et al. 2018).

4 HOB in hydrogen-enriched soil ecosystems

The majority of facultative H_2-oxidizing chemolithotrophs can only recycle H_2 in elevated concentrations, as their threshold for H_2 exceeds the atmospheric concentration. Fluxes with elevated H_2 are produced during fermentation, nitrogen (N_2) fixation, and geochemical processes (Greening et al. 2021). And H_2-enriched soil niches including agricultural soils especially rhizosphere in the vicinity of nodulated plants, volcanic or geothermal soils, and termite mounds. Such organisms are predestined to utilize H_2 that is transiently available in biologically relevant concentrations. It is well known that soils rapidly become anoxic when they are waterlogged. This can lead to transient production of H_2 when soil microbes shift to fermentation (Schwartz et al. 2013). Elevated concentrations of H_2 are favorable for HOB using low-affinity hydrogenases. Low-affinity HOB may be more niche-restricted than those oxidizing atmospheric H_2 (Greening et al. 2016). The composition of microflora in H_2-enriched soil niches is displayed in Fig. 5.

4.1 Agricultural soil

4.1.1 Distribution

The N_2 fixation process is responsible for accumulating H_2 (~20,000 ppmv) in legumes rhizosphere of agricultural soil, e.g., soybean-, clover-, vetch-like nodules surroundings (Pumphrey et al. 2011; Xu et al. 2021). Obligate aerobic soil rhizobia, e.g., *Azotobacter* unavoidably produces H_2 as a byproduct during N_2 fixation (Eq. 6) (Constant et al. 2009; Noar and Bruno-Bárcena 2016).

$$N_2 + 8H^+ + 16ATP + 8e^- \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi \quad (6)$$

This source of H_2 has a seasonal variation affecting nodule numbers and root nutrient availability (Conrad and Seiler 1980; Meredith et al. 2017; Li et al. 2018). Wild-type *Azotobacter* recaptures this H_2 with uptake hydrogenase (Hup^+), thus preventing waste of energy and reducing equivalents (Schink and Schlegel 1978). As major legume crops nodulated with rhizobia lack uptake hydrogenases (Hup^-), substantial amounts of H_2 (~240,000 L H_2 per hectare per growing season) are released in the surrounding N_2-fixing nodules, enhancing the number/activity of HOB, including *Proteobacteria, Actinobacteriota, and Bacteroidetes*

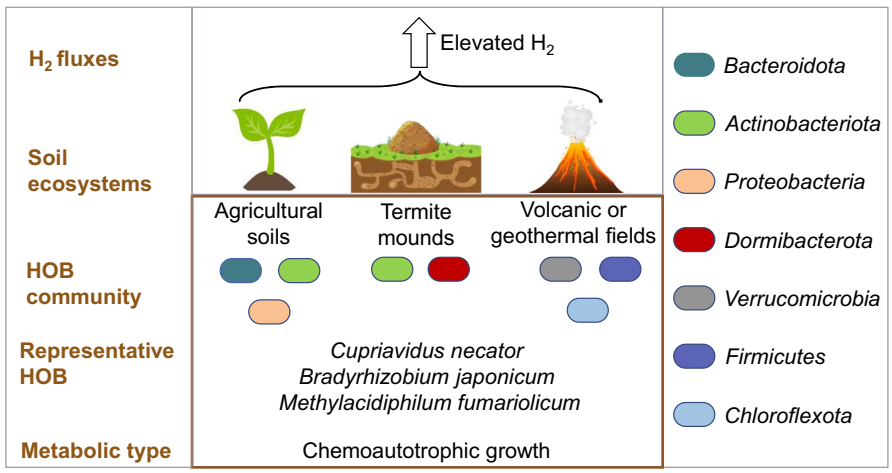


Fig. 5 Microbial composition of HOB in agricultural soil, volcanic or geothermal fields with elevated levels of H_2 fluxes

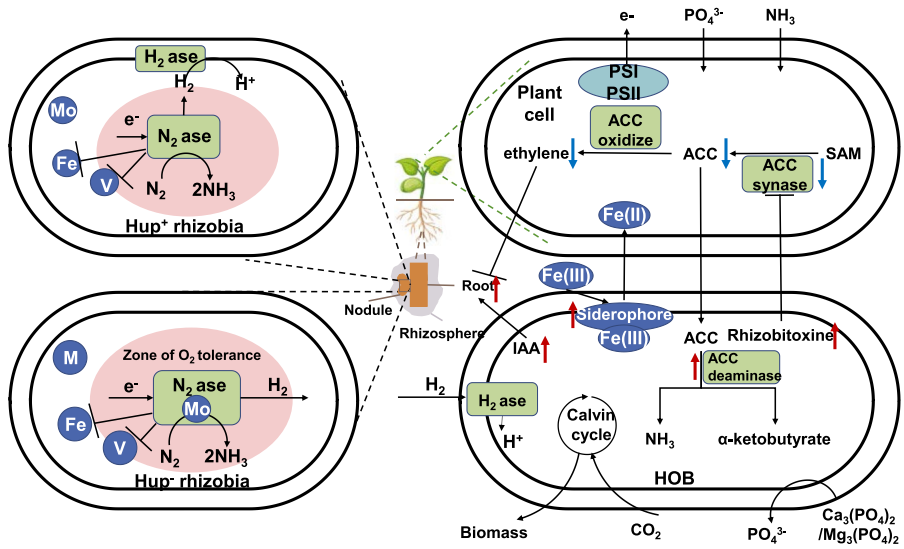


Fig. 6 Interaction between HOB, plant, and rhizobia in the rhizosphere. N_2 ase: nitrogenase with Mo as central cofactors in Hup + and Hup- rhizobia; H_2 ase: hydrogenases that recycle the H_2 produced by nitrogenase; Hup^+/Hup.$^-$: with/without hydrogenases; IAA, indole-3-acetic acid; SAM: S-adenosyl-L- methionine; ACC, 1-aminocyclopropane-1carboxylic acid; PSI, photosystem I; PSII, photosystem II. Stimulating and inhibitory interactions mediated by species traits or metabolites are marked with red and blue arrows. The upside-down T-bars represent inhibition/ blockage (Conrad and Seiler 1980; Golding and Dong 2010; Noar and Bruno-Bárcena 2016; Li et al. 2019; Lin et al. 2022)

(Dong and Layzell 2001; Golding et al. 2012; Abdellatif et al. 2017).

4.1.2 Cooperation with rhizobia

The rhizosphere of agricultural soil is one of the richest niches with microbial diversity in which plants secrete secondary metabolites around roots (Philippot et al. 2013; Prashar et al. 2014). Colonization of HOB, which exists as rhizobacteria, plays a crucial role in shaping rhizobia–host interactions in many legumes such as clover, soybeans, peas, and alfalfa (Han et al. 2020). The interaction between plants, rhizobia, and HOB can be described as follows: rhizobia in root nodules as one of the best-known symbiotic diazotrophs converts atmospheric N_2 into ammonia mediated by nitrogenase (Bruslind 2019a, b). At the same time, root nodules secrete root exudates as nutrients for rhizobia. Since the uptake hydrogenase system have low activity in many rhizobia, H_2 is produced as a byproduct of nitrogenase which claims about 5–6% energy of the crops' net photosynthesis (Dong and Layzell 2001; Dong et al. 2003; Islam et al. 2016; Wang et al. 2020a). HOB especially low-affinity species are recruited within 3 to 4.5 cm of the nodule and may have a competitive advantage over other rhizobacteria in microaerophilic soil conditions (Abdellatif et al. 2017). HOB have the potential to protect the nitrogenase from inactivation by oxygen, thus improving the energy efficiency of the rhizobium-legume symbiosis (Schink and Schlegel 1978). Thus, the interaction of rhizobia and HOB is chiefly an interplay between two enzyme systems: hydrogenase and nitrogenase (Fig. 6).

4.1.3 Ecological roles of plant growth promotion

Although not a nutrient, HOB are promising biofertilizers and plant growth-promoting rhizobacteria (PGPR) which could maintain crop productivity, reducing chemical fertilizer use (Golding et al. 2012). HOB promote the growth of N_2-fixing soybean, alfalfa plants nodulated with Hup- rhizobia, and lentils nodulated with *Rhizobium leguminosarum* (Hup +). Besides, HOB can promote root nodulation due to promoting carbon deposition (Maimaiti et al. 2007). It was reported that an extra 25 kg of soil carbon was fixed for a legume fixing 200 kg N_2 per hectare (Osborne et al. 2010). Most of the HOB isolates living in the vicinity of H_2-producing nodules were members of the genera *Mycobacterium*,

Table 2 Summary of plant promotion in H_2-oxidizing bacteria

Place	Phylum	Species	Beneficial traits	References
Non-nodulated soybean, non-leguminous barley, spring wheat, and canola	–	–	the dry weight of soybean, barley, spring wheat, and canola increased by 15–48%, while the tiller numbers increased by 36–48% induced by H_2	(Dong et al. 2003)
Spring wheat	–	–	H_2-treated soil increases spring wheat's dry weight by 20.6% after 35 days compared to air-treated soil. Antibiotic treatment decreases dry weight	(Irvine et al. 2004)
The rhizosphere of clover and alfalfa	*Beta-, Gammaproteobacteria*	–	H_2 treatment selectively enhance the populations of PGPRs based on FISH	(Stein et al. 2005)
Field soil adjacent to the Hup– nodules	*Bacteroidetes*	*Flavobacterium Johnsonian*	increasing ACC deaminase activity	(Jiamila Maimaiti et al. 2007)
Pb and Cd contaminated-soil	*Betaproteobacteria*	*Burkholderia sp.*	heavy metal and inorganic phosphate solubilization	(Jiang et al. 2008)
Soybean root systems and microcosm experiments	*Actinobacteriota*	*Streptomyces sp., Pseudonocardia sp., and Mycobacterium sp.*	H_2 treatment did not change the overall community composition, but the relative abundance of HOB single-member increased detected by T-RFLP	(Osborne et al. 2010)
Barley and spring wheat (cereal non-leguminous crops)	*Bacteroidetes*	*Flavobacterium Johnsonian*	increase plant height, tiller numbers, and head density with the inoculation of *F. Johnsonian* compared to controls	(Golding et al. 2012)
Semiarid areas where lentil is grown	*Gammaproteobacteria*	*Acinetobacter sp.*	siderophore production, ACC deaminase production, antifungal activity	(Abdellatif et al. 2017)
	Betaproteobacteria	*Variovorax paradoxus*	phosphorus solubilization, siderophore, and IAA production, antifungal activity production	
	Actinobacteriota	*Rhodococcus sp. Mycobacterium sp. Curtobacterium sp.*	enhanced siderophore, IAA, ACC deaminase production, and antifungal activity	
Soybean	*Alphaproteobacteria*	*Bradyrhizobium japonicum*	co-inoculation of rhizobia *B. japonicum* and root-colonizing *P. putida* improved soybean growth, nodulation, nutrient uptake, and soil enzymes such as protease, acid/alkaline phosphomonoesterase in the drought-stressed soil	(Jabborova et al. 2021)
	Gammaproteobacteria	*Pseudomonas putida*		

Acinetobacter, *Curtobacterium*, *Variovorax*, *Flavobacterium,* and *Burkholderia*. The research on promoting effects concerning HOB is summarized in Table 2.

HOB surrounding legumes promote plant growth through direct mechanisms including increasing phosphorus cycling and production of indole-3-acetic acid (IAA). To be specific, HOB applied to cultivated soil enhance the solubilization of calcium or magnesium phosphate which come from applied phosphorus fertilizers. One typical strain with this trait is the *Variovorax paradoxus* (Abdellatif et al. 2017). Produced phytohormone IAA increased plant biomass and nutrient uptake. HOB also foster plant growth indirectly through the synthesis of siderophores (iron chelation), decreasing aminocyclopropane-1carboxylic acid (ACC) synthase and increasing ACC deaminase, and producing certain biocontrol compounds to suppress the growth of phytopathogens. These three metabolites are related to ethylene or pathogens. HOB produce a siderophore that can chelate with Fe^{3+} on bacterial membrane, then reduces it to Fe^{2+}, improving the level of plant-available iron (II) (Sultana et al. 2021). Iron availability to plants rather than pathogens inhibits the growth and sporulation of pathogens (Abdellatif et al. 2017). *Bradyrhizobium japonicum* and *Burkholderia* were found to be rhizobitoxine-positive strains. Rhizobitoxine inhibits the ACC synthase enzyme, leading to a decrease in ACC, the precursor of ethylene. Combined with an increase in the activity of ACC deaminase which hydrolyzes ACC to a-ketobutyrate and ammonia, ethylene production is reduced (Jiamila Maimaiti et al. 2007; Golding and Dong 2010). Through decreasing ethylene levels in the host plant or antifungal properties against common root pathogens, HOB finally promote nodulation in legumes and protects plant productivity against abiotic stress like drought, salinity, and nutrient deprivation (Abdellatif et al. 2017; Wang et al. 2020b). The ecological role of plant promotion is displayed in Fig. 6.

4.2 Volcanic soil

Elevated H_2 was also detected in the volcanic and geothermal sites characterized by high temperatures (approximately 60 °C at the surface and rise with depth), acidic pH (3–5), as well as fluctuating O_2 concentrations, and substrate availability (Pumphrey et al. 2011; Picone et al. 2020). Besides H_2, uprising hydrothermal gases consist mainly of CO_2 and varied CH_4 and H_2S (Picone et al. 2020). These geothermal features provide a suitable niche for the growth of HOB, mainly *Verrucomicrobia* and *Firmicute* phylum (Hogendoorn et al. 2020; Picone et al. 2020). Methanotrophs *Methylacidiphilum fumariolicum SolV* (*Verrucomicrobia*) and carboxydotrophic *Kyrpidia spormannii* (*Firmicute*) are two special HOB taxa discovered in geothermal soils (Mohammadi et al. 2017; Hogendoorn et al. 2020; Schmitz et al. 2020).

4.3 Termite mounds

Soil-derived termite mounds are minor H_2-evolving habitats of fermenting bacteria. In the termite mounds, the H_2 concentration can be as high as 10,000 ppm due to carbohydrates fermentation in the gut of arthropods including termites, particularly performed by *Clostridia*. The hydrogen-rich environment is conducive to the survival and growth of thermoacidophilic HOB dominated by diverse *Actinobacteriota* and *Dormibacterota* (Chiri et al. 2021).

5 HOB in soil ecosystems exposed to atmospheric hydrogen

Soil ecosystems exposed to atmospheric H_2 include temperate soils such as forests, wetland, grassland, dryland, and upland, as well as oligotrophic deserts (Fig. 7). The biogeochemical cycle of atmospheric H_2 depends on biological, geochemical, and anthropogenic contributions (Greening et al. 2015b). Despite the high levels of internal gas cycling due to rapid ex-situ rates and endogenous gas production, the tropospheric H_2 maintains low net in-situ gas fluxes of 0.553 ppmv (Bay et al. 2021). The H_2 oxidation process in soils constitutes the primary sink in the global biogeochemical H_2 cycle and mediates the net loss of 70 million tons of H_2 per year, three-quarters of which can be largely attributed to aerobic HOB (Greening et al. 2015b; Islam et al. 2020; Leung et al. 2022). Aerobic HOB employ high-affinity group 1 h and 2a [NiFe]-hydrogenases to scavenge and oxidize H_2 gas that has diffused into the subsurface soil from the atmosphere. This process has a significant role in

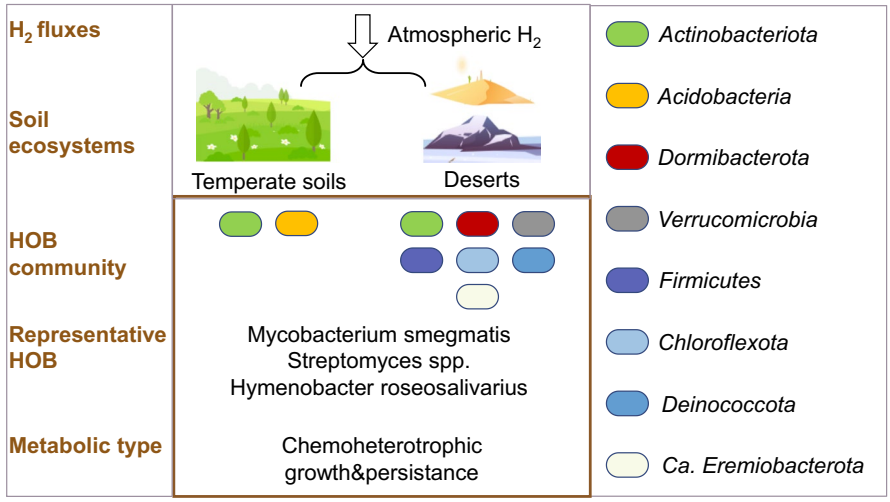


Fig.7 Microbial composition of HOB in temperate soils, hot and cold deserts with atmospheric levels of H_2 fluxes

mediating atmosphere-soil interaction and fueling the survival or growth of HOB, thereby sustaining the productivity of soil ecosystems (Liot and Constant 2016; Jordaan et al. 2020; Greening et al. 2021).

5.1 Temperate soil

5.1.1 Distribution

Aerobic high-affinity HOB could be omnipresent in temperate soils by scavenging atmospheric H_2. The process of atmospheric H_2 chemosynthesis also extends to nuclear power plant sites. In the soil near the Cernavoda nuclear power plant site, HOB were also detected converting D_2 (Deuterium Gas) to DHO (a molecule of semi-heavy water). Besides, HOB were detected in soil with antibiotics pollutants (Willms et al. 2020). The most reported are *Actinobacteriota* (Constant et al. 2008) and *Acidobacteriota* (Fig. 7) (Greening et al. 2015a; Andrew T. Giguere et al. 2021), mainly possessing group 1 h and 2a [NiFe]-hydrogenases to serve as the sink of atmosphere H_2 (Islam et al. 2019a; Greening et al. 2021).

5.1.2 Competition between HOB

In temperate soils such as forests, grassland, wetland, farmland, and meadow, high- and low-affinity HOB with biphasic kinetics may coexist in the soil environment, inducing intense resource competition between HOB (Greening et al. 2021). The competition among HOB populations for H_2 and nutrition induces the disappearance of some low-affinity populations and makes high-affinity HOB dominant in temperate soils. On the other hand, it was observed that many HOB coexisted with methane, ammonia, and nitrite oxidizers encoding hydrogenase lineages that used more niche substrates (Greening et al. 2016, 2021). Interactions between HOB and these specialist taxa remain to be studied.

5.1.3 Maintaining atmosphere H_2 balance

A large proportion of microbes especially sporulators adopt dormant strategies in temperate soil to reduce energy expenditure. Still, energy is required for basic cell maintenance and environmental sensing (Greening et al. 2015b; Leung et al. 2022). Due to its high energy content, low activation energy, and high diffusibility (diffusion coefficient 4×10^{-9} m^2 s^{-1}), atmospheric H_2 serves as an energy source and electron donor, especially HOB during periods of nutrient deprivation in low-carbon and energy-starved soils or hypoxia environments (Conrad 1996; Greening et al. 2015b, 2016, 2021; Islam et al. 2019b, 2020; Bay et al. 2021). With the ubiquity of stress-tolerant group 1f (Hyo), 1 h (Hhy), 1 l (Hyl), 1 m (Hhm), 2a (Huc) [NiFe]-hydrogenases, HOB contribute to the relative stability, productivity, and biodiversity of soil populations, and most importantly, maintaining atmospheric pH_2 at about 0.553 ppm (Golding and Dong 2010; Greening and Grinter 2022). Taken together, chemolithoheterotropic HOB persist in temperate soils using ubiquitous H_2 as electron donors to drive aerobic

respiration, sustaining the stability of microbial community structures, which was the main sink of the global H_2 sink. Atmospheric H_2 oxidation will also increase the metabolic flexibility of streptomycetes, which is likely to be crucial during their transitions from free-living, epiphytic, and endophytic states (Greening and Cook 2014).

5.2 Desert soil

5.2.1 Distribution

Deserts can be classified as sub-humid, semi-arid, arid, and hyper-arid according to the precipitation to evapotranspiration ratio (P/ET) (Zomer et al. 2008; Bay et al. 2018). Many deserts globally were investigated for the community dynamics of HOB, including hot deserts distributed in Asia (e.g., Taklimakan Desert), America (e.g., Atacama Desert), and Africa (e.g., Kalahari Desert), as well as cold deserts distributed in Antarctic, Arctic, Asia (e.g., Tibetan Plateau), America (e.g., Yellowstone) (Spear et al. 2005; Bahl et al. 2011; Ji et al. 2017; Schulze-Makuch et al. 2018). Microbial life inhabiting deserts faces physical/chemical pressures, including nutrient and moisture availability, and temperate and UV radiation variation across multiple spatial scales, which curtail cellular and metabolic activities (Bay et al. 2018; Ray et al. 2022). Chemoheterotrophs may enter a reversible dormant state of low metabolic activity globally in deserts (Lennon and Jones 2011). Due to inherent heterogeneity in terms of physical–chemical characteristics, and nutrient bioavailability, the evolution and, assembly of microflora vary between desert soils (Bahl et al. 2011; Caruso et al. 2011). For instance, the community structure has undergone a strong selection of a *Pseudonocardia sp.* (*Actinobacteriota*) in the hyper-arid and high-elevation Atacama Desert (Lynch et al. 2014). Phylogenetically and functionally diverse atmospheric-H_2-oxidizing organisms were detected in deserts globally dominated by phyla *Actinobacteriota*, *Verrucomicrobia*, *Chloroflexota*, *Dormibacterota, Ca. Eremiobacterota*, *Deinococcota,* and *Firmicutes* (Leung et al. 2020; Ray et al. 2022).

5.2.2 Cooperation with cyanobacteria

Face with harsh physicochemical conditions, two possible pathways of primary production sustain heterotrophic energy and organic carbon of dormant chemoheterotrophs: photosynthetic and chemosynthesis processes (Jordaan et al. 2020). The underlying balance between the two pathways along aridity gradients remains to be discussed (Bay et al. 2018). In photosynthetic taxa, carbon fixation genes (RuBisCO) are mainly encoded by *Cyanobacteria* (type IB) as well as rhodopsins to transduce solar energy into a proton motive force through the CBB cycle (Jordaan et al. 2020). *Cyanobacteria* perform oxygenic photosynthesis and fix CO_2 into biomass, supporting primary production in deserts (Ciani et al. 2021). They colonize fissures and biological soil crusts to withstand the UV radiation and desiccation of desert ecosystems (Bay et al. 2018). Despite these advantages as primary producers, *Cyanobacteria* was observed to have a limited capacity to drive carbon fixation and electron transport due to a low average relative abundance (Ray et al. 2022). Other phototrophs are also excluded or restricted to lithic niches due to aridity (Ji et al. 2017; Bay et al. 2018). Conversely, HOB appear to have genetic advantages by aerobically respiring atmospheric H_2 with high-affinity hydrogenases (Ray et al. 2022). Metagenomic and RT-PCR data from several samples in hot and polar deserts identified the expression of the genes encoding type IE RuBisCO (support hydrogenotrophic growth) and high-affinity hydrogenases (groups 1h, 1l, and 2a) (Ji et al. 2017; Jordaan et al. 2020; Ortiz et al. 2021; Ray et al. 2022). Soil communities are thus maintained primarily by H_2 rather than by solar energy. However, during periods of precipitation in deserts, moisture availability would stimulate *Cyanobacteria* and, in turn, accounts for a lower relative abundance of desiccation-tolerant HOB. Hydration modulates dominated community members shifting from dormant chemoheterotrophs to actively growing photoautotrophs in deserts (Jordaan et al. 2020). Consequently, phototrophy and H_2 scavenging are likely to co-occur in many Antarctic sites, with the balance shift depending on physicochemical factors (Jordaan et al. 2020). Besides the co-occurrence of *Cyanobacteria* and HOB, *Cyanobacteria* produce H_2 during N_2 fixation or using NAD(P)H as reductant catalyzed by 'bidirectional' [NiFe]-hydrogenases (hoxEFUYH) under anaerobic fermentative conditions during oxygenic photosynthesis, which may be in favor of inducing HOB (Friedrich et al. 2011; Lupacchini et al. 2021).

5.2.3 Energy and carbon supply

Compared to temperate soils, the microbial community structure of desert soils harbors similar phyla but specialized species, probably driven by physicochemical factors (Ji et al. 2017). In deserts, bacterial communities face nutrient deprivation as plants are generally limited to moss and lichens. Therefore, the metabolic potential of the microbial communities to recycle nutrients and catabolize plant-derived organic compounds is low, and a wide range of detected heterotrophs encoding carbohydrate-active enzymes was inactive (Ray et al. 2022). Most heterotroph communities adopt a dormant state to adapt to the harsh environmental conditions, the energy and carbon needs of which are sustained by atmospheric chemosynthesis, especially H_2 oxidation by aerobic HOB (Lynch et al. 2014; Bay et al. 2018). H_2 readily diffuses air-filled soil pores and through cell membranes in deserts, allowing HOB to outcompete other autotrophs utilizing water-soluble substrates such as ammonia (Yang et al. 2019). In deserts, HOB harboring terminal oxidase genes play a role in energy harvesting through the aerobic respiratory chain (Jordaan et al. 2020). Thermodynamic modeling suggested that the in-situ maintenance energy of aerobic heterotrophs was 10^{-17} to 10^{-19} W per cell and the energy derived from H_2 oxidation (2.2×10^{-15} W per H_2-oxidizing cell) was theoretically sufficient to sustain persistence (Bay et al. 2021). Different from hydrogenotrophic bacteria which exclusively scavenge H_2 for energy acquisition, special HOB such as *Pseudonocardia* spp. can generate mass through H_2-driven CO_2 fixation using high-affinity 1 h [NiFe]-hydrogenases (Grostern and Alvarez-Cohen 2013). After the addition of H_2 in desert soil, the assimilation of 14C-labelled CO_2 was enhanced by an average of twofold, thereby confirming the capacity of carbon acquisition by the HOB community (Ji et al. 2017). Taken together, HOB help shape the biodiversity of soil communities by providing an alternative basis to solar or geological energy sources and dependable carbon sources to support dormant communities, and in turn, enhances the resilience of desert ecosystem functions (Greening and Grinter 2022).

6 Conclusion and outlook

6.1 Conclusions

This review summarizes the diversity of HOB in physiology and distribution. And the advances in isolation and identification techniques are systematically summarized. Aerobic soil HOB are classified as (1) chemolithoautotrophic HOB including some *Proteobacteria* and rhizobia, which encode low-affinity hydrogenases (the group 1d [NiFe]-hydrogenases) that sustain growth by recycling elevated H_2 in the rhizosphere of agricultural soils, volcanic or geothermal soils, and termite mounds; (2) chemoheterotrophic HOB including *Mycobacterium smegmatis* and *Streptomycetes spp.* (Actinobacteria) use atmospheric H_2 to maintain survival in temperate soils and deserts. Various HOB are currently identified through cultivation-dependent-, molecular-, meta-omics, and mass spectrometry techniques. Functionally distinct hydrogenases in soil niches allow HOB to adopt various metabolic and ecological strategies. The expression of hydrogenases and structures of HOB taxa may depend on the availability of electron donors and acceptors, and the environmental variables such as soil nutrient pools, temperature, and moisture. Finally, the paper accesses the HOB-biota interactions in different soil ecosystems. Competition occurs between high- and low-affinity HOB in temperate soils, and the total activity of the community maintains atmospheric H_2 balance. In the rhizosphere of agricultural soils and desert soils, HOB cooperate with non-HOB communities including Rhizobia (Hup^-) and *Cyanobacteria*, promoting plant growth and supplying carbon and energy, respectively.

6.2 Outlook

(1) Many HOB have revealed unique flexibility in hydrogenases resulting in hydrogen metabolic versatility, which allows them to combat diverse soil niches. Although the structures and genes encoding hydrogenases are widely recognized in soil ecosystems, the role of hydrogenases in cells and communities remains unresolved (Greening et al. 2016). With the combination of cultivation-dependent experiments and genetics and biochemical analysis of hydrogenases, multiple molecular and meta-omics techniques, fur-

ther study is expected to build a strong linkage between hydrogenase structure, differential regulation, and cell functioning.

(2) In addition to H_2 and O_2 concentration, nutrients availability, moisture, and temperature, other environmental variables such as land use, climatic conditions, and soil depth also drive the H_2 oxidation activity, distribution of hydrogenases, microbial community diversity, and composition (Osborne et al. 2010; Khdhiri et al. 2018; Bay et al. 2021)., Knowledge of responses and constraints of HOB to ecological factors allows a deeper understanding of the key biogeochemical process of H_2 oxidation and biomass production across different soil niches (Osborne et al. 2010). In future field trials, the potential ecological factors can be optimized to shape a more productive, efficient, competitive HOB group, thereby promoting crop growth and producing more yield of cultivated land or shaping biodiversity in oligotrophic soil ecosystems.

(3) In termite mounds, volcanic soils, and deep-sea hydrothermal vents where the exchange of H_2 and metabolites is abundant, microbiota include fermenters producing H_2, other hydrogenotrophic bacteria (methanotrophs, sulfate-, and Fe(III)-reducing bacteria) and chemolithoautotrophic bacteria utilizing other inorganic energy sources (H_2S, CO) or CH_4 (Greening et al. 2016). HOB are in symbiotic associations with gut arthropods in termite guts and with invertebrates in hydrothermal vents to produce biomass (Petersen et al. 2011; Chiri et al. 2021). Intensive studies should understand the interactions between HOB–microbiota, which is expected to construct a functionality-stable consortium with enhanced community-level CO_2 fixation and energy utilization ability and the abatement of CH_4 and CO oxidation activities.

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Declarations

Conflict of interest The authors declare that no conflict of interest exists in the submission of this manuscript, and the manuscript is approved by all authors for publication.

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