Acidobacteria are active and abundant members of diverse atmospheric H₂-oxidizing communities detected in temperate soils

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Abstract

Significant rates of atmospheric dihydrogen (H₂) consumption have been observed in temperate soils due to the activity of high-affinity enzymes, such as the group 1h [NiFe]-hydrogenase. We designed broadly inclusive primers targeting the large subunit gene (hhyL) of group 1h [NiFe]-hydrogenases for long-read sequencing to explore its taxonomic distribution across soils. This approach revealed a diverse collection of microorganisms harboring hhyL, including previously unknown groups and taxonomically not assignable sequences. Acidobacterial group 1h [NiFe]-hydrogenase genes were abundant and expressed in temperate soils. To support the participation of acidobacteria in H₂ consumption, we studied two representative mesophilic soil acidobacteria, which expressed group 1h [NiFe]-hydrogenases and consumed atmospheric H₂ during carbon starvation. This is the first time mesophilic acidobacteria, which are abundant in ubiquitous temperate soils, have been shown to oxidize H₂ down to below atmospheric concentrations. As this physiology allows bacteria to survive periods of carbon starvation, it could explain the success of soil acidobacteria. With our long-read sequencing approach of group 1h [NiFe]-hydrogenase genes, we show that the ability to oxidize atmospheric levels of H₂ is more widely distributed among soil bacteria than previously recognized and could represent a common mechanism enabling bacteria to persist during periods of carbon deprivation.

Introduction

Soil bacteria consume molecular hydrogen (H₂) from the Earth’s atmosphere and serve as the main sink in the global biogeochemical H₂ cycle as demonstrated in field and laboratory-based investigations [1–6]. Hydrogen oxidation in soil follows biphasic kinetics with both high-affinity (Kₘ < 100 nM) and low-affinity (Kₘ > 1000 nM) enzyme activities [7, 8]. The ability to oxidize H₂ stems from the presence of specialized metalloenzymes, called hydrogenases, that catalyze the conversion of H₂ to protons and electrons [9]. Bacteria with low-affinity have been known for decades [10] and are believed to grow on high concentrations of H₂ produced in microniches, such as N₂-fixing root nodules [11]. In contrast, bacteria that consume atmospheric H₂ (~0.53 ppmv) [5] remained elusive until recently [12].

While soil microorganisms harbor a range of hydrogenases that catalyze H₂ oxidation under oxic conditions [9, 10, 13–16], it is thought that the group 1h [NiFe]-hydrogenases are primarily responsible for (sub-) atmospheric H₂ oxidation [13, 14, 17], as seen with several previously isolated actinobacteria [9, 12] that consume atmospheric H₂ to conserve energy during persistence [17–19]. Actinobacteria are
abundant in soils based on culture-independent studies [9, 14, 20] and were thought to be primarily responsible for atmospheric H₂ oxidation [14, 16].

Recent genomic and metagenomic investigations, along with pure culture work, have identified additional bacteria harboring group 1h [NiFe]-hydrogenases within the phyla Acidobacteria, Proteobacteria, Planctomycetes, Chloroflexi and Verrucomicrobia [14, 21–23]. Notably, Acidobacteria are one of the most abundant soil phyla with relative abundances in 16S rRNA libraries ranging from ca. 20 to 40% in temperate soils such as forests, grasslands and pasture soils [24]. They constitute a large and phylogenetically distinct phylum [25, 26] that harbors, diverse physiologies [27]. Two thermophilic acidobacterial strains were previously shown to consume atmospheric levels of H₂, due to the presence of high-affinity [NiFe] hydrogenases [21, 28]. A recent large-scale comparative genome analysis of acidobacteria also identified the genes encoding the large and small subunits of the group 1h [NiFe]-hydrogenase (hhyL and hhyS, respectively) in genomes of various mesophilic soil acidobacteria, along with the necessary maturation and accessory genes [29]. Yet, it remained unknown whether mesophilic acidobacteria can scavenge H₂, which are highly abundant in temperate soils where atmospheric H₂ consumption was previously reported [30–32].

As more and more taxonomic groups have been identified to harbor group 1h [NiFe]-hydrogenases, it was our goal to design broadly inclusive primers for long-read sequencing that allow the investigation of diverse group 1h [NiFe]-hydrogenase communities. Sequencing of almost the complete large subunit gene further enables improved phylogenetic placement and identification of the amplified genes from soil samples. Using this new primer pair, we demonstrate that group 1h [NiFe] hydrogenases are widespread across many phyla, including previously unidentified groups. In addition, we illustrate that mesophilic acidobacteria are prevalent and active members of the group 1h [NiFe] hydrogenase-harboring community in H₂-consuming temperate soils and are capable of atmospheric H₂ consumption. This work therefore reveals new mediators in the biogeochemically and ecologically important process of atmospheric H₂ oxidation, and supports growing evidence that trace gases might be a universal energy source for bacterial persistence.

Materials and methods

Screening publicly available genomes and MAGs

Publicly available genomes and metagenome-assembled genomes (MAGs) (n = 175509, November 2018) were screened for the presence of hydrogenase large subunit genes using pfam model PF00374.19, as well as models constructed to be more sensitive to [NiFe] lineages 1–4 from HydDB [33]. For lineage-sensitive models, [NiFe] hydrogenases contained in HydDB were separated into the four major lineages [1–4] and models were constructed de novo. Amino acid sequences for each lineage were extracted from HydDB, clustered into centroids using usearch [34] (–sortbylength and –clustersmallmem –id 0.85) and aligned using MAFFT [35]. The resulting alignments were trimmed using trimAl with setting:automated1 [36] and models were constructed using hmmbuild from hmmer3 [37]. [NiFe] hydrogenases were identified in genomes and MAGs using hmmbuild (e-value<0.001). For all hidden Markov model (hmm) hits, the putative genes were back-screened against the Pfam-A database to verify that pfam model PF00374.19 was the best matching Pfam. All putative group 1 [NiFe]-hydrogenase large subunit genes (hhyL) were extracted and further screened using the HydDB online classifier [33]. CheckM was used to estimate completeness, contamination and heterogeneity of the genomes based on lineage-specific markers [38]. The taxonomy of MAGs containing the hhyL gene with a completeness of >50% was determined using the Genome Taxonomy Database (https://gtdb.ecogenomic.org). Duplicate copies of hhyL in a genome were removed and scored as one. The amino acid sequences derived from these hhyL genes were used to explore the phylogeny and confirm the taxonomic assignment of the full-length sequences retrieved in this study. Sequences were aligned with MUSCLE [39] and phylogenetic trees were generated using FastTree JTT + CAT model, along with estimating FastTree confidence [40].

Soil sample collection and nucleic acid extraction

Soil samples were collected from (a) a mature beech forest (Fagus sylvatica L.), ca. 40 km southwest of Vienna, Austria (more details can be found in [41]) collected in summers of 2012, 2013, 2014; (b) a managed grassland from the agricultural research station (AREC) in Raumberg-Gumpenstein, Austria (49°29‘37”N, 14°06’10”E; more details on the site can be found in [42]) collected in the summer of 2018; (c) the rhizosphere of Arrhenatherum elatius (tall oatgrass) grown at this aforementioned managed grassland, collected in the summer of 2018; and (d) biological soil crusts (of ~5 mm thickness) from the central Negev Desert, Israel (30°47‘N, 34°46’E; more details on the site can be found in [43]) collected in the summer of 2017. DNA and RNA were extracted from ca. 0.4–0.5 g of soil using a modified bead-beating protocol in the presence of a CTAB buffer and phenol as previously described [44]. Samples were purified using OneStep™ PCR Inhibitor Removal Kit (Zymo, Irvine, CA, USA) and quantified using the Qubit
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determine when the
allows one to vary total carbon (here supplied as glucose) to
was monitored by measuring the optical density at 600 nm.
(0.38) and stationary (OD600nm ranging from 0.53) phase by centrifugation at 10000 RPM for 10 min to allow pelleting of these cells that produce extracellular material. Harvested cells were concentrated 10-fold, resuspended in 10 mL carbon-free VSB medium and transferred to 110 mL serum vials sealed with butyl rubber stoppers. Headspace was flushed with synthetic air (Messer Gas, Bad Soden, Germany) and supplemented with ~20 ppmv H2 (Linde Gas, Dublin, Ireland). Strains were incubated at 24 °C on an orbital shaker (ca. 130 RPM) for 7 days. Headspace samples were periodically sampled and the H2 concentration was determined via a Trace GC Ultra (Thermo Scientific, Austria) with a pulse discharge detector (PDD). This GC has the ability to accurately detect H2 down to concentrations of 0.5 ppmv. A gas chromatograph with a pulsed discharge helium ionization detector (model TGA-6791-4U-2, Valco Instruments Company Inc. (VICI, Houston, TX, USA)) was used for sub-atmospheric H2 concentrations (ca. 0.1 to 0.5 ppmv) as previously described [22]. To ensure an oxygenated headspace during these incubations, oxygen concentrations were monitored and were never lower than 18% (v/v) (Fig. S2). Hydrogen consumption controls were run on uninoculated medium, heat-killed Acidobacteriaceae bacterium KBS 83 cells and an acidobacterial strain (Terribi-
globus roseus KBS 63) that does not contain group 1h [NiFe]-hydrogenase genes [29] over a period of 24 h with starting H2 concentrations of ~80 ppmv to ensure any

**Bacterial strains and growth conditions**

Acidobacterial strains (*Acidobacteriaceae* bacterium KBS 83 [56] and *E. aggregans* [57]) were grown using a defined vitamins and salts medium, VSB-6 [56, 58], with 5 mM glucose as the carbon source. The use of a defined medium allows one to vary total carbon (here supplied as glucose) to determine when the final yield (as measured by optical density at 600 nm) reduced to the proportional decrease in glucose concentration. To determine carbon-limiting conditions, strains were grown in the aforementioned defined medium in differing glucose concentrations (5 mM and 10 mM glucose). Carbon-limiting conditions were defined when the cellular density was proportional to the amount of carbon provided. They were incubated on an orbital shaker (ca. 130 RPM) under aerobic conditions at 24 °C. Growth was monitored by measuring the optical density at 600 nm.

**Primer design and RT-qPCR**

Total RNA was extracted from cultures using a modified standard bead-beating protocol [44] with one-round of bead beating and acidified phenol/chloroform/isoamyl alcohol (pH 4.5). Extracts were purified with the Turbo DNA-free kit according to the manufacturer’s protocol. RNAs were normalized to 3 ng µL⁻¹ and ca. 30 ng were used for cDNA synthesis using SuperScript IV Reverse Transcriptase, following the manufacturer’s protocol.

Quantitative PCR (qPCR) was performed on a C1000 Touch thermocycler equipped with a CFX96 Real Time System in combination with iQ SYBR-Green qPCR Assay (Biorad, Hercules, CA, USA). Primers were designed for the large (*hhyL*) and small (*hhyS*) subunits of the group 1h [NiFe]-hydrogenase of *E. aggregans* and *Acidobacteri-
aceae* bacterium KBS 83 targeting the same region of the respective gene, along with the homolog of the *hhyS* as was done previously [21]. All primers are listed in Table S1. Standard curves were constructed with 10-fold serial dilutions of genomic DNA of each respective strain, typi-
cally ranging between 10⁶ to 1 copy. The qPCR assay was performed in 20 µl volume containing the following components: 10 µl of SYBR Green Supermix (Biorad, Hercules, CA, USA), 0.4 ng µl⁻¹ BSA (Thermo Fisher Scientific, Waltham, MA, USA), 0.2 µM (E. aggregans) or 1 µM (*Acidobacteriaceae* bacterium KBS 83) of each primer and 1–5 µl of cDNA template. The program used was: 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 65 °C (*hhyS*, *hhyL* for *Acidobacteriaceae* bacterium KBS 83 were run at 68 °C and 58 °C, respectively) for 30 s for annealing and 72 °C for 1 min for extension. The expression of the large and small subunit genes of the group 1h [NiFe]-hydrogenase was assessed using these newly designed primer pairs upon normalization to the 16S rRNA gene using the above assay but with an annealing temperature of 68 °C. Melting curves were generated between 65 °C and 95 °C. Data were processed and analyzed using the CFX Manager software (Biorad, Hercules, CA, USA) and data were logtransformed to determine fold-increase. Data on the speci-ficity of the qPCR assay can be found in Fig. S1.

**H2 consumption and hydrogenase activity measurement assays for acidobacterial cultures**

Briefly, cells were harvested in exponential (OD600nm ranging from 0.25–0.38) and stationary (OD600nm ranging from 0.50–0.53) phase by centrifugation at 10000 RPM for 10 min to allow pelleting of these cells that produce extracellular material. Harvested cells were concentrated 10-fold, resuspended in 10 mL carbon-free VSB medium and transferred to 110 mL serum vials sealed with butyl rubber stoppers. Headspace was flushed with synthetic air (Messer Gas, Bad Soden, Germany) and supplemented with ~20 ppmv H2 (Linde Gas, Dublin, Ireland). Strains were incubated at 24 °C on an orbital shaker (ca. 130 RPM) for 7 days. Headspace samples were periodically sampled and the H2 concentration was determined via a Trace GC Ultra (Thermo Scientific, Austria) with a pulse discharge detector (PDD). This GC has the ability to accurately detect H2 down to concentrations of 0.5 ppmv. A gas chromatograph with a pulsed discharge helium ionization detector (model TGA-6791-4U-2, Valco Instruments Company Inc. (VICI, Houston, TX, USA)) was used for sub-atmospheric H2 concentrations (ca. 0.1 to 0.5 ppmv) as previously described [22]. To ensure an oxygenated headspace during these incubations, oxygen concentrations were monitored and were never lower than 18% (v/v) (Fig. S2). Hydrogen consumption controls were run on uninoculated medium, heat-killed Acidobacteriaceae bacterium KBS 83 cells and an acidobacterial strain (*Terri-
globus roseus* KBS 63) that does not contain group 1h [NiFe]-hydrogenase genes [29] over a period of 24 h with starting H2 concentrations of ~80 ppmv to ensure any
potential consumption activity would be sufficiently high for detection (Fig. S3).

The enzyme kinetics ($V_{\text{max}}$, $K_{\text{m(app)}}$) of the group 1h [NiFe]-hydrogenase of pure cultures were determined using similar methods as described in [17] using gas chromatography. Briefly, cells were harvested as described above, concentrated and resuspended in carbon-free VSMB medium with various concentration of H$_2$ (ranging from 0 to 1000 ppmv). Consumption was monitored via gas chromatography over a period of 120 h. H$_2$ uptake rates were determined at each respective concentration and normalized to mg of protein using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Enzyme kinetics for the Michaelis–Menten kinetics, non-linear least squares method was determined in R [59], while the Hanes-Woolf plot was calculated manually.

**Soil H$_2$ consumption assays**

Soil samples were collected from aforementioned mature beech forest, managed grassland and desert biological soil crusts to explore H$_2$ consumption. Briefly, approximately 1–2 grams of soil (or of natural dry soil crust) was incubated in a sealed 110 mL serum bottle, flushed with synthetic air and supplemented with ~20 ppmv H$_2$. H$_2$ consumption was monitored in the temperate soils using a Trace GC Ultra (Thermo Scientific, Austria) with a PDD-type detector down to a concentration of 0.5 ppmv; sub-atmospheric H$_2$ concentrations (ca. 0.1–0.5 ppmv) were determined on a gas chromatograph with a pulsed discharge helium ionization detector (PDHID) (model TGA-6791-W-4U-2, Valco Instruments Company Inc. (VICI)) as described above. All H$_2$ measurements of biological soil crust samples were performed using the VICI gas chromatograph with a PDHID detector.

To determine the $K_{\text{m(app)}}$ of H$_2$ for the beech forest soil, we incubated 1–2 grams of soil in a sealed 110 mL serum bottle. The headspace was flushed with synthetic air and supplemented with H$_2$ ranging from 10 to 1000 ppmv, as higher concentrations are needed to estimate $K_{\text{m(app)}}$ and $V_{\text{max}}$. The headspace was sampled over 24 h and uptake kinetics were calculated using the Michaelis–Menten kinetics, non-linear least squares method was determined in R [59] and normalized to gram dry soil.

**Results**

**Grassland and forest soils harbor active and diverse communities of atmospheric H$_2$-oxidizing bacteria**

We measured bacterial H$_2$ consumption in soils from a beech forest, a managed grassland and a desert biological soil crust. The desert soil crust consumed H$_2$ very slowly over the course of two weeks to sub-atmospheric levels (Fig. 1a). In contrast, the forest and managed grassland soils rapidly consumed H$_2$ to sub-atmospheric levels over a 24-h period (Fig. 1a). Differences between H$_2$ uptake amongst the soils are reflected in the contrasting rate constant ($k$) values (Fig. 1a). There was no significant difference in the estimated rate constant values between the temperate soils. The apparent Michaelis constant, $K_{\text{m(app)}}$, was estimated to be 33 ± 12 nM (Fig. 1b in the beech forest soil); this soil had a significantly ($p$ value < 0.05) higher rate constant value when compared to the biological soil crust.

To reveal the microorganisms mediating this H$_2$ uptake, we designed degenerate primers to target the group 1h [NiFe]-hydrogenase large subunit genes ($hhyL$) for long-read amplicon sequencing of the different soil communities. These primers were designed to encompass the diversity of $hhyL$ sequences across ten different phyla. We amplified and sequenced $hhyL$ genes from total community DNA extracted from the managed grassland (bulk and rhizosphere) and forest soils, along with biological soil crusts. Overall, diverse $hhyL$-encoding communities were observed across the soils, with 2403 OTU$_{95}$ clusters (95% cut-off) identified in total. The designed primers proved highly specific for the group 1h [NiFe]-hydrogenase large subunit genes ($hhyL$) for long-read amplicon sequencing of the different soil communities. These primers were designed to encompass the diversity of $hhyL$ sequences across ten different phyla. We amplified and sequenced $hhyL$ genes from total community DNA extracted from the investigated soils (Fig. 2) alongside those that we retrieved from previously published genomes and metagenome-assembled genomes (Figs. 2 and 3). Bacteria within these temperate soils that encoded group 1h [NiFe]-hydrogenase sequences were affiliated with Acidobacteria, Actinobacteria, Chloroflexi, Nitrospirae, Planctomycetes, Proteobacteria (Alpha-, Beta- and Delta-) and Verrucomicrobia (Fig. 2a, b). In contrast, the biological soil crusts harbored group 1h [NiFe]-hydrogenase sequences predominantly affiliated with Actinobacteria and Chloroflexi (Fig. 2a, b).

There was an additional deep-branching cluster of sequences containing group 1h [NiFe]-hydrogenases of the
diverse origin, such as members of the phyla Actinobacteria, Chloroflexi, Bacteroidetes, Acidobacteria, Proteobacteria and Euryarchaeota (Fig. 2b, “Distant group 1h cluster”). Although these sequences were all classified as a group 1h [NiFe]-hydrogenase based on the HydDB [33], they appear to be distantly related to the other, main branch of the tree (Fig. 2b). Approximately 244 OTU representatives of the long-read sequences were placed in this cluster; these sequences were found across all soil samples, but were most prevalent in the biological soil crust samples (ca. 10–27%). In addition, there were various OTU representatives that did not group in clusters that contain reference sequences (Fig. 2b, gray dots).

For an evaluation of the newly designed long-read primer pair, we compared its coverage with the coverage of previously published short-read primer pairs ([NiFe]-244F/568R and [NiFe]-1129F/1640R [18, 52]. These short-read primers led to similar patterns of alpha- and beta-diversity (Fig. S5) compared to the long-read primers (Figs. 1c, d and S4). However, the long-read primer pair captured a breadth of diversity exceeding those attained with the short-read primers (Fig. S6). More specifically, the newly designed long-read primer pair captured an additional group of actinobacterial sequences (Fig. S6c, top-most Actinobacteria cluster), along with putative members in the Deltaproteobacteria, Nitrospira and the deep-branching cluster of group 1h [NiFe]-hydrogenases of diverse origin (Fig. S6c, “Distant...
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various phyla, including the Acidobacteria, were expressed in the temperate soils (Supplementary Information SI-1, Figs. S9–10). Acidobacteria isolated from temperate forest and grassland soils oxidize atmospheric H2 during carbon limitation

The above culture-independent inferences suggest that members of the Acidobacteria may be important mediators of atmospheric H2 oxidation in temperate soils. As such, we investigated the gene expression and activity of the group 1h [NiFe]-hydrogenase present in two mesophilic acidobacterial isolates, the grassland soil bacterium Acidobacteriaceae bacterium KBS 83 [56] and forest soil bacterium Edaphobacter aggregans [57]. In addition to containing hhyL and hhyS [29], both organisms also possess an additional copy of hhyS ca. 3,600 bp upstream of the structural and maturation genes, as observed in P. methylaliphatogenes [21] (Supplementary Information SI-2).

Transcription of both the large (hhyL) and small (hhyS) subunits of the hydrogenase was upregulated during carbon-limitation (Fig. 4a, d). We used defined media to determine the conditions that induced stationary phase due to carbon-limitation in both strains (Fig. S11). Under stationary phase conditions, the transcription of the hhyL gene (normalized to the 16S rRNA gene) was upregulated by ~125-fold in Acidobacteriaceae bacterium KBS 83 (Fig. 4a) and ~3.5-fold in E. aggregans (Fig. 4d) compared to exponential growth. In both organisms, transcription of the hhyS gene was not detected during exponential phase (< 2 copies per ng cDNA), but was detected during stationary phase (Fig. 4a, d). The apparent differential expression of the hhyS and hhyL genes in exponential phase may be attributed to different promoter regions; potential different promoter regions were computationally identified across the structural genes of Acidobacteriaceae bacterium KBS 83, which presumably also have different transcription factors (Fig. S12). The expression of the hhyS homolog was detected in Acidobacteriaceae bacterium KBS 83, but not in E. aggregans (Supplementary Information SI-2).

We subsequently tested whether these strains consume atmospheric H2 under carbon-limiting conditions. Both strains consumed H2 from levels of ~20 ppmv to a concentration of 0.25 ± 0.03 ppmv (Acidobacteriaceae bacterium KBS 83) and 0.36 ± 0.06 ppmv (E. aggregans) after 175 h (Fig. 4b, e). H2 was only consumed by carbon-limited stationary cells; no H2 oxidation was observed in cells exponentially growing on the defined medium (Fig. S13) or in E. aggregans cultures grown to stationary phase on an undefined medium (presumably due to non-carbon-limiting conditions) (Fig. S14). Likewise, no H2 was consumed in the uninoculated medium, heat-killed controls, or in an acidobacterial strain lacking the group 1h [NiFe]-hydrogenase (Terriglobus roseus) (Fig. S3). The kinetic parameters of the group 1h [NiFe]-hydrogenases were determined on whole cells of strains Acidobacteriaceae bacterium KBS 83 and E. aggregans. The apparent half-saturation constant (Km(app)) measured on cells of Acidobacteriaceae bacterium KBS 83 was 173 ± 63 nM H2 with a saturating rate (Vmax(app)) of 0.27 ± 0.03 µmol H2 mg protein−1 h−1 (Fig. 4c). The Km(app) for H2 uptake by E. aggregans was 95 ± 31 nM with a Vmax(app) of 6.16 ± 1.06 nmol H2 mg protein−1 h−1 (Fig. 4f); similar values were observed using another model (Table S5). These kinetic parameters of the group 1h [NiFe]-hydrogenases were determined on whole cells of strains Acidobacteriaceae bacterium KBS 83 and E. aggregans.
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Fig. 4 Expression, activity, and kinetics of the enzymes mediating atmospheric H₂ oxidation in two acidobacterial strains isolated from temperate soils. Acidobacteriaceae bacterium KBS 83 and Edaphobacter aggregans. a, d Growth curves of the strains over time (days) (x-axis) and expression levels (inset) of the group 1h [NiFe]-hydrogenase structural subunit genes (large, hhyL; small, hhyS) during exponential and stationary phase. Arrows depict the growth phases in which cells were harvested for gene expression investigations (gray arrow, exponential phase; black arrow, stationary phase). During this experiment, H₂ consumption was measured on stationary phase cells (black arrows) and in a parallel experiment on exponential phase cells (Fig. S13), as H₂ consumption assays required the entire biomass of such an experiment. b, e H₂ consumption of stationary phase stage cells of each respective strain; x-axis depicts the start of measurements for H₂ consumption after harvesting cells from growth curves of panels a, d. Dashed lines represent atmospheric H₂ concentrations (~0.53 ppmv), whereas red points depict the heat-killed controls for each respective strain. The final sub-atmospheric H₂ measurement for each strain was performed on a gas chromatograph with a pulsed discharge helium ionization detector (model TGA-6791-W-4U-2, Valco Instruments Company Inc.); this measurement is indicated by an asterisk. Over the course of the experiment, we observed a slight decrease in H₂ for our medium control (8%). Even when this loss is accounted for in the final sub-atmospheric measurement, the concentration is still below atmospheric levels of H₂. (0.27 ppmv for Acidobacteriaceae bacterium KBS 83 and 0.39 ppmv for E. aggregans). Additional controls can be found in Fig. S3. c, f Apparent kinetic parameters of H₂ oxidation for the strains based on whole-cell assays. Best-fit curves were determined using the Michaelis–Menten non-linear regression model; similar values were observed using Hanes–Woolf plots (Table S5).

Discussion

Our genomic surveys and the targeted hhyL amplicon sequencing of soil samples add to the growing evidence that group 1h [NiFe]-hydrogenases appear to be widespread in numerous taxonomic groups (Fig. 2). The genomic survey identified new groups, namely members of the Deltaproteobacteria and Nitrospira, that harbor group 1h [NiFe]-hydrogenase genes, in addition to Acidobacteria, Actinobacteria, Bacteriodetes, Chloroflexi, Planctomycetes, Proteobacteria (Alpha- and Beta-) and Verrucomicrobia (Figs. 2 and S8) in accordance with previous surveys [9, 10, 14, 16]. These phyla are commonly found in soils with varying relative abundances [64]; however, it should be noted that with the exception of the Acidobacteria and Actinobacteria, group 1h [NiFe]-hydrogenases are present in less than 3% of genomes from each of these phyla (Fig. S8). Furthermore, all of the mentioned phyla are amplifiable with our newly designed primers (Fig. 2), thus allowing future investigations to explore the distribution of these groups across environments using long-read amplicon sequencing.
Our newly designed group 1h [NiFe]-hydrogenases primers not only capture the diversity of previously established group 1h [NiFe]-hydrogenases primers [18, 52], but also additional sequence diversity across edaphically different soils (Fig. S6). In comparison to these other primers, phylogenetic analysis revealed the presence of additional clusters as well as OTUs without reference sequences (Fig. S6, in gray), the latter suggesting the presence of putative novel hydrogenases. We suggest that the use of long-read \textit{hhyL} sequences could allow for improved phylogenetic placement and identification of the amplified sequences from environmental samples, along with the use of the Evolutionary Placement Algorithm implemented in RAxML using a base tree built with not only publicly available \textit{hhyL} sequences (mostly stemming from isolates) but also with \textit{hhyL} genes identified in genomes and MAGs of uncultured organisms (Fig. S8). As such, we have added an additional primer pair to the toolbox of exploring the group 1h [NiFe]-hydrogenases in environmental samples. Although it is unclear if it captured the complete diversity of atmospheric H\textsubscript{2}-oxidizers harboring group 1h [NiFe]-hydrogenases in the investigated soils, it captured some putative novel sequences and previously undetected groups (Fig. 2). These findings will aid future work to attribute which community members and enzyme lineages are responsible for the biogeochemically important process of atmospheric H\textsubscript{2} oxidation.

Biological soil crusts exhibited the lowest diversity estimates (Fig. 1c), being dominated by \textit{hhyL} sequences assigned to \textit{Actinobacteria}, \textit{Chloroflexi} and the “Distant group 1h cluster” (Fig. 2b), and the slowest H\textsubscript{2} consumption (Fig. 1a, \(k = 0.0051\)). Temperate soil libraries had higher diversity estimates, especially in the managed grassland soil (Fig. 1c), and contained sequences related to the \textit{Actinobacteria}, \textit{Acidobacteria}, \textit{Planctomycetes}, \textit{Proteobacteria} and \textit{Verrucomicrobia} (Fig. 2). These soils exhibited faster H\textsubscript{2} consumption (Fig. 1a; managed grassland \(k = 0.1752\), forest soil \(k = 0.2495\)). Follow-up investigations will be necessary to reveal the contributions of these different groups to H\textsubscript{2} oxidation (also including hydrogenases other than the group 1h-type), but preliminary sequencing of cDNA shows a diverse community actively transcribing \textit{hhyL} genes in the temperate soils (Fig. S10).

Many of the \textit{hhyL} sequences detected by using the new primer pair clustered with the two acidobacterial strains tested here for H\textsubscript{2} consumption (\textit{Acidobacteriaceae} bacterium KBS 83 and \textit{E. aggregans}), in addition to other mesophilic acidobacteria such as \textit{Acidobacteriaceae} bacterium KBS 96, \textit{G. mallensis}, and “Ca. Solibacter usitatus” Ellin6076”. All of these strains are described as aerobic heterotrophs [56, 57, 61, 65], which is consistent with the group 1h [NiFe]-hydrogenase being both oxygen-tolerant [66] and linked to the aerobic respiratory chain [10]. The acidobacterial group 1h [NiFe]-hydrogenase sequences from genomes and MAGs formed a distinct clade (Figs. 2 & 3) together with those from the \textit{Planctomycetes}. Some of these \textit{Planctomycetes}-affiliated sequences were generated from MAGs and, as such, this clustering could be a result of poor assemblies, incompleteness or high contamination of MAGs. Yet it is unlikely as this cluster also contains sequences stemming from pure cultures (such as \textit{Singulisphaera acidiphila}) (Fig. 3).

We demonstrate that mesophilic acidobacteria are capable of scavenging H\textsubscript{2} in pure culture (Fig. 4). Although previous work has shown that two thermophilic acidobacterial strains scavenge atmospheric H\textsubscript{2} either due to a group 1h or 1f [NiFe]-hydrogenase [21, 28], only very few sequences with an identity of \(\geq 97\%\) could be detected in the
NCBI database and of those identified, were primarily from thermophilic environments (Fig. S15). Therefore, investigating the \( \text{H}_2 \)-oxidation capability in representative mesophilic strains inhabiting temperate soils was essential. With the data presented in this study on mesophilic acidobacteria, this is first report of a mesophilic bacterium outside the \textit{Actinobacteria} [9, 20, 67] being capable of atmospheric \( \text{H}_2 \) oxidation via a group 1h [NiFe]-hydrogenase. We were further able to detect expressed acidobacterial \textit{hyl} in temperate soils (Fig. S10), illustrating that acidobacteria are active in the soils that consume \( \text{H}_2 \) (Fig. 1a). This is ecologically significant given mesophilic acidobacteria are abundant across temperate soils [68], which together comprise ~20–30% of terrestrial environments [69]. Further investigations are warranted to determine the acidobacterial contribution to \( \text{H}_2 \) consumption in temperate soils, for instance in comparison to the well-studied actinobacteria. The two investigated strains, \textit{Acidobacteriaceae} bacterium KBS 83 and \textit{E. aggregans}, exhibit mid to high-affinity \( \text{H}_2 \) uptake kinetics comparable to those measured for other bacteria harboring group 1h [NiFe]-hydrogenases (Fig. 5, asterisks). The whole soil communities within the beech forest soil also exhibited high-affinity \( \text{H}_2 \) uptake kinetics (Fig. 1b), suggesting that \( \text{H}_2 \) is predominantly being oxidized by bacteria expressing high-affinity enzymes (i.e., group 1h [NiFe]-hydrogenases). This is congruent with previous surveys showing these enzymes are the most prevalent hydrogenases in grassland and forest soils [14].

The \( K_{\text{m(app)}} \) of the investigated mesophilic soil acidobacteria (95 and 172 nM) was higher compared to the thermophilic acidobacterium \textit{P. methylaliphilatogenes} (35 nM), but in line with the higher-end of model high-affinity \( \text{H}_2 \)-oxidizing bacteria such as representatives in the \textit{Actinobacteria} (Fig. 5). Yet the \( K_{\text{m(app)}} \) of the investigated strains was lower than the Chloroflexi strain \textit{Thermomicrobium roseum} that consumes \text{H}_2 from both geological and atmospheric sources [22] (Fig. 5). This further demonstrates that group 1h [NiFe]-hydrogenases spanning both the high- and mid-affinity \( K_{\text{m(app)}} \) values are capable of using atmospheric concentrations of \text{H}_2. The \( V_{\text{max}} \) rate for \textit{Acidobacteriaceae} bacterium KBS 83 were ca. 5-fold higher than that observed for \textit{P. methylaliphilatogenes} [21], but 2 to 6-fold lower than those observed for \textit{T. roseum} [22] and \textit{Mycobacterium smegmatis} [17]. In contrast, the \( V_{\text{max}} \) rate of \textit{E. aggregans} was orders of magnitude lower than the aforementioned strains. This observed variation in \( V_{\text{max}} \) within the strains used in this study and those previously published [17, 21] suggest there is high variability among group 1h [NiFe]-hydrogenases. Furthermore, the relative contributions of group 1h [NiFe]-hydrogenases to atmospheric \text{H}_2 uptake in-situ remain unclear, as does to what extent duration of carbon starvation or other growth-limiting conditions influences group 1h [NiFe]-hydrogenases expression and potential activity in soil environments.

Our data suggest that mesophilic acidobacteria use atmospheric \text{H}_2 to adapt to carbon starvation, which is in accordance with other soil strains [16, 17]. The structural genes encoding the hydrogenases were highly upregulated by both species in stationary-phase, carbon-starved cultures compared to exponentially growing, carbon-replete cultures (Fig. 4a, d). Likewise, activity was only observed in carbon-starved cultures (Figs. 4b, e and S14). Exponential bacterial growth is a state rarely found in nature; rather in many ecosystems (such as soil), bacteria enter a non-replicative persistent state [70] for extended periods of time [71]. It was estimated that up to 80% of the soil microorganisms at a given time [72] will be in such a state, often referred to as dormancy. Hydrogen has previously been proposed as a possible universal energy source for survival [73]. It was then proposed more specifically that the use of group 1h [NiFe]-hydrogenases facilitate the ability to scavenge atmospheric \text{H}_2 to sustain aerobic respiration during periods of starvation in actinobacteria [17] and thermophilic acidobacteria [21, 28]. In this study, we further extend this working hypothesis to encompass mesophilic acidobacteria. It is likely that the oxidation of atmospheric \text{H}_2, as a ubiquitous, diffusible, high-energy substrate, enables mesophilic acidobacteria to meet maintenance energy needs during persistence [10, 18]. \text{H}_2-scavenging acidobacteria could have a selective advantage in periods of carbon depletion and therefore an increased likelihood to persist in the soil. Although group 1h [NiFe]-hydrogenase genes could only be detected in ca. 7% of 745 investigated acidobacterial genomes (Fig. S8), this may reflect the lack of adequate representations of \textit{Acidobacteria} in our public databases relative to the breadth of diversity found in nature. It is also plausible to assume that there are other physiologies that allow persisting periods of carbon starvation, such as atmospheric carbon monoxide oxidation as suggested recently for actinobacteria [74] and chloroflexi [22].

Overall, the finding that mesophilic acidobacteria and likely other diverse microorganisms in soil can oxidize atmospheric \text{H}_2 has important implications for both atmospheric chemistry and microbial ecology. These bacteria potentially contribute to the major sink of the global \text{H}_2 cycle. Moreover, atmospheric \text{H}_2 scavenging is hypothesized to contribute to bacterial persistence, with theoretical estimates predicting that atmospheric \text{H}_2 provides the necessary maintenance energy for \( 10^7 \) to \( 10^8 \) bacteria per gram of soil [10, 18]. This physiology could, therefore, aid in maintaining populations and genotypes, ultimately sustaining soil microbial biodiversity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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