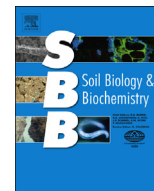
Soil Biology & Biochemistry 85 (2015) 1–9

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio



Soil carbon content and relative abundance of high affinity H_2-oxidizing bacteria predict atmospheric H_2 soil uptake activity better than soil microbial community composition

Mondher Khdhiri [a,1], Laura Hesse [a,1], Maria Elena Popa [b], Liliana Quiza [a], Isabelle Lalonde [a], Laura K. Meredith [c], Thomas Röckmann [b], Philippe Constant [a,*]

[a] Institut National de la Recherche Scientifique-Institut Armand-Frappier, 531 boulevard des Prairies, Laval, Québec H7V 1B7, Canada
[b] Institute for Marine and Atmospheric Research Utrecht, Utrecht University, Princetonplein 5, 3584 CC Utrecht, The Netherlands
[c] Earth Environmental System Science, Stanford University, 473 Via Ortega, Stanford, CA 94305-4216, USA

A R T I C L E I N F O

Article history:
Received 5 August 2014
Received in revised form
23 February 2015
Accepted 26 February 2015
Available online 11 March 2015

Keywords:
Gaseous exchanges
Hydrogen
Molecular biogeochemistry
Trace gas

A B S T R A C T

Soil–atmosphere exchange of H_2 is controlled by gas diffusion and the microbial production and oxidation activities in soil. Among these parameters, the H_2 oxidation activity catalyzed by soil microorganisms harboring high affinity hydrogenase is the most difficult variable to parameterize because it is influenced by many unknown edaphic factors that shape microbial community structure and function. Here we seek to formulate a model combining microbiological and physicochemical variables to predict the H_2 oxidation rate (u) in soil. Soil sample replicates collected from a grassland and three forests exhibited different H_2 oxidation potentials. We examined the microbial community structure based on ribotyping analysis, the relative abundance of high affinity H_2-oxidizing bacteria (HOB) estimated by qPCR and soil physicochemical characteristics as predictors for u. A single linear regression parameterized by total carbon content and a multiple linear regression using total carbon content and HOB relative abundance in soil explained 66 and 92% of the variance in u, respectively. Microbial community composition based on 16S rRNA gene pyrosequencing profiles was not a reliable predictor for u. Indeed, we found that HOB are members of the rare biosphere, comprising less than 1% of total bacteria as estimated by qPCR. We confirmed this relationship of u with total carbon content and HOB by an independent soil survey of 14 samples collected from maize monocultures, grasslands, deciduous forests and larch plantations. Observations made from both soil surveys thus were combined to build a predictive model for u parameterized with total carbon content and HOB relative abundance. Our results show that molecular biogeochemistry is a potential approach to improve performance of classical H_2 surface flux models which estimate u empirically without considering variation in HOB distribution and activity in soil.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Molecular hydrogen (H_2) is present at trace levels in the atmosphere, with a typical background mole fraction of 530 ppbv (Novelli et al., 1999). Combustion of fossil fuels and biomass, and oxidation of methane and non-methane hydrocarbons are the main sources of H_2, summing to 80 Tg yr^{-1} global annual emissions (Constant et al., 2009; Ehhalt and Rohrer, 2009; Pieterse et al., 2013). It is likely that the atmospheric burden of H_2 has remained unaltered since the 1990s (Novelli et al., 1999). This balance may be attributed to soil microorganisms, which today account for about 80% of the total sink of atmospheric H_2. Considering the fact that H_2 reacts with the hydroxyl radical (OH), the cleansing molecule in the atmosphere responsible for the removal of most atmospheric methane, it is important to verify whether the biological sink of atmospheric H_2 will be vulnerable, resistant or resilient to ongoing global change. One key issue is to better constrain the environmental factors influencing the distribution and activity of high affinity H_2-oxidizing bacteria (HOB) that thrive in aerobic soil.

* Corresponding author. Tel.: +1 450 687 5010x4117.
E-mail address: Philippe.Constant@iaf.inrs.ca (P. Constant).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.soilbio.2015.02.030
0038-0717/© 2015 Elsevier Ltd. All rights reserved.

Previous efforts to build predictive models of H_2 soil uptake activity have parameterized the activity as a function of ecosystem type and/or biophysicochemical parameters. In a meta-analysis of H_2 soil uptake measurements reported in the literature, H_2 dry deposition rates varied between 0.01 and 0.15 cm s^{-1}, and were generally higher in temperate forest ecosystems than temperate grassland and agricultural lands (Ehhalt and Rohrer, 2009). This observation was further supported by an extensive investigation of the impact of land-use change on atmospheric trace gas turnover where native rainforest acted as a stronger sink for H_2 than rainforest sites converted to pasture and hardwood plantation (Pendall et al., 2010). Two main process-based parameters have been found to explain the variance of H_2 dry deposition velocity, namely gas diffusion in soil and HOB metabolic activity (Yonemura et al., 2000; Smith-Downey et al., 2008). HOB activity is mainly influenced by temperature, availability of growth substrates, microbial community structure and water potential in soil. The dependence of microbial H_2 soil uptake activity on soil moisture and temperature has been analyzed extensively (Ehhalt and Rohrer, 2011), leading to the development of a two-layer model parameterized with soil porosity, soil water content and a fixed number of HOB to predict H_2 deposition velocity (Ehhalt and Rohrer, 2013). One key limitation for the application of this model to predict H_2 uptake in soil is a scaling factor referring to the number of metabolically active HOB in soil. Indeed, the model assumes a fixed number of HOB for a given soil class, and empirical adjustments of this variable were necessary to improve model agreement with experimental values (Ehhalt and Rohrer, 2013). The empirical adjustments reflect documented variability of H_2 oxidation activity potential in soil encompassing a broad range of physicochemical characteristics as well as the ecophysiology of HOB. Specifically, laboratory experiments have demonstrated that high affinity H_2 oxidation activity is restricted to resting cells, while suitable growth conditions inhibit their uptake activity (Constant et al., 2008; Meredith et al., 2013). In addition, a soil survey of the *hhyL* gene encoding the large subunit of the high affinity [NiFe]-hydrogenase that is responsible for HOB oxidation of atmospheric H_2 revealed large variation in the abundance of this functional group between 10^6 and 10^8 presumptive cells per gram of soil (Constant et al., 2011b; Greening et al., 2014). Finally, the high affinity hydrogenase is unevenly distributed in *Actinobacteria* and to a lesser extent in *Chloroflexi*, *Acidobacteria* and *Proteobacteria* demonstrating a broad range in term of cell-specific H_2 oxidation activity – from 0.03 to 18 amol cfu^{-1} h^{-1} (Constant et al., 2011b). This highlights the fact that variation in microbial community structure could influence H_2 soil uptake rate.

With the exception of one study demonstrating a relationship between H_2 oxidation rate (u) with substrate-induced respiration, nitrate concentration and pH in soil, very few attempts have been made to identify a proxy for u (Gödde et al., 2000). In this study, we seek to combine soil molecular and physicochemical datasets to predict u in soil. We tested the hypothesis that the relative abundance of certain taxonomic groups of bacteria and HOB could be used as predictors for H_2 uptake activity in soil.

2. Materials and methods

2.1. Soil samples

Soil samples were collected in the Netherlands from a grassland site near the Cabauw tall tower research site (51°58′N, 4°55′E) and from the Speuld forest (52°13′N, 5°39′E). At the Speuld forest, samples were taken from three monoculture plots: beech (about 60 years old), mature spruce (about 60 years old) and young spruce (about 25 years old). Representative soil samples of the A-horizon were obtained by collecting three independent samples per ecosystem type, resulting to 12 samples in total. Samples were stored at 4 °C for six months before they were dried at 20 °C for 48 h and homogenized (2 mm sieve). Soil water content was adjusted to 20% water holding capacity (whc) with sterile water and subsamples (15 $g_{(dw)}$ per replicate) were transferred into 500 ml Gibco® glass bottles (nominal volume) fitted with foam plugs to allow gaseous exchanges between soil and atmosphere, while avoiding microcosm contamination with airborne particles. Soil microcosms were then transferred to an environmental chamber (MLR-350, Sanyo, Osaka, Japan) and incubated 3 days in the dark, at 25 °C and 50% relative air moisture. This incubation was necessary for the activation of HOB following soil drying and homogenization treatments. Indeed, preliminary experiments consisting to monitor H_2 oxidation rate in soil microcosms over a period of 7 days showed that H_2 uptake activity reach a plateau after 2–3 incubation days (data not shown). Selected physicochemical parameters were analyzed in soil after incubation. Soil pH was analyzed in soil:water suspensions (1:2.5) and soil water content was measured using the standard gravimetric method. Soil nutrients were analyzed in external laboratory facilities (INRS Centre Eau, Terre et Environnement, Canada). Phosphorus and potassium were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES) after acid extraction, while total soil carbon and nitrogen contents were determined using an elemental analyzer.

A second soil survey was undertaken to validate the observations made from the original 12 samples. The origin (*i.e.* land-use type, site location) and physicochemical characteristics of these 14 additional soil samples are summarized in Table S1. Briefly, two samples were collected from the Harvard forest (samples Harvard-F1, Harvard-F2) previously investigated for H_2 flux measurements (Meredith et al., 2014). Ten samples were collected in grasslands (samples CLE1-Grass, CLE2-Grass), deciduous forest (samples VER-F3, WEN-F3), maize monocultures (samples VER-A3, WEN-A2, CLE-A1) and larch plantations (samples VER-M3, WEN-M3, CLE-M3) from three locations on the south shore of the St. Lawrence River, located about 40 km (VER; town of Verchères), 90 km (WEN; town of Saint-Cyrille-de-Wendover) and 130 km (CLE; town of Saint-Claude) from Montreal city. These three sites are tree nurseries for spruce, larch and pine established by the ministère des ressources naturelles-Québec (MRNQ) for seed production to support reforestation programs. The landscape of these three sites is a mosaic encompassing a broad range of ecosystem types arranged over a relatively small area (<1 km^2). Fifteen years ago, the MRNQ converted part of the original agricultural areas of the three sites to tree plantations, leaving some parcels for maize production as well as unseeded lands that led to the emergence of a natural deciduous forest and grasslands. Finally, two soil samples were collected from a deciduous forest (sample IAF-F1) and a grassland (sample IAF-Grass) in the vicinity of the INRS-Institut Armand-Frappier on the north shore of River of the Prairies. These additional samples were processed using the same procedure as the first soil survey. In addition to soil total carbon, total nitrogen and water content analyses, soil texture was determined by the hydrometer method and the particle size distribution was used to identify soil samples textural class (Elghamry and Elashkar, 1962).

2.2. H_2 soil uptake activity

H_2 oxidation activity measurements were performed using a gas chromatography assay. Briefly, soil microcosm foam plugs were replaced with gastight caps equipped with butyl septa. A defined volume of air mixture containing 525 ± 10 ppm H_2 (GST-Welco, Pennsylvania, U.S.A.) was injected to the static headspace

of the microcosms, resulting in a H_2 level of 2–3 ppmv. Headspace samples (10 ml) were collected with a Pressure Lok® gastight glass syringe (VICI® Precision Sampling Inc., Baton Rouge, Louisiana, USA) and injected through the injection port of a gas chromatograph equipped with a reduction gas detector (ta3000R, Ametek Process Instruments®, Delaware, U.S.A.). The first-order H_2 oxidation rate was calculated by integrating the H_2 mole fraction time series measured over a 1-h period, using at least five H_2 concentration points for data integration. The reproducibility of the H_2 analyses was assessed before each set of experiments by repeated analysis of certified H_2 standard gas mixture (2.13 ppmv ±5%, GST-Welco, Pennsylvania, USA), and standard deviations were <5%. No significant H_2 uptake was observed for blank measurements of empty microcosms, and the amount of soil (15 g) relative to the headspace volume (720 ml) precluded diffusion limitation of H_2 uptake activity measurements as preliminary experiments showed proportional H_2 oxidation rate as a function of the amount of soil in the microcosm using 10, 25, 50 and 75 g soil samples (data not shown). The gas chromatographic assay was repeated every incubation day, and microcosms were capped with a foam plug and transferred back to the environmental chamber after the measurements. Because of the occurrence of simultaneous H_2 production and consumption activities in nature and their dependence on temperature and moisture, rates of H_2 oxidation presented in this study must be considered as potential H_2 uptake activities.

2.3. DNA extraction and internal standard

Genomic DNA was extracted from soil microcosms at the end of the 3-day incubation, using the Fast DNA®Spin Kit for Soil (MP Biomedicals®, Solon, Ohio, U.S.A.). Soil subsamples (0.2–0.5 g) were weighed prior the extraction procedure and genomic DNA was eluted in 100 μl of nuclease-free water. An internal standard DNA (IS) was used to normalize qPCR data to account for the presence of humic acids and other PCR inhibitors. The IS developed by Deer et al. (Deer et al., 2010) was synthetized by GenScript USA Inc. (Piscataway, New Jersey, U.S.A.) and maintained in the plasmid pUC57 transferred to *Escherichia coli* DH5α. PCR-amplified IS was added to the DNA extracts, leading to an equivalent concentration of 10^5 IS μL^{-1}, which is in the same magnitude as the abundance of *hhyL* typically retrieved from soil extracts (Constant et al., 2011a). DNA aliquots were stored at −20 °C.

2.4. qPCR assays

The abundance of HOB was estimated using a *hhyL* qPCR assay (Constant et al., 2011a), while the bacterial biomass in soil was estimated by the 16S rRNA gene qPCR assay designed by Fierer et al. (Fierer et al., 2005). The reactions were performed using 5 μl of diluted genomic DNA (1:500). Absolute quantifications were normalized using the IS (Deer et al., 2010; DeCoste et al., 2011), which increased *hhyL* and 16S rRNA gene abundance data values by 1–2 orders of magnitude. The three qPCR assays were based on standard curves prepared by using triplicate 10-fold dilutions of PCR-amplified standard DNA. Genomic DNA of *Streptomyces avermitilis* and *Burkholderia xenovorans* LB400 served as template for *hhyL* and 16S rRNA gene standard DNA, respectively. PCR products were purified (E.Z.N.A. Cycle Pure Kit, Omega Bio-Tek®, Norcross, Georgia, U.S.A.) and quantified using the Quantifluor™ dsDNA System (Promega, Madison, Wisconsin, U.S.A.) according to the instructions of the manufacturers. Standard curves encompassing 10^2 to 10^8 copies μL^{-1} of standard DNA were prepared and displayed linear relationship between the signal and the logarithm copy number with reaction efficiencies of 0.81 ($r^2 = 0.99$), 0.99 ($r^2 = 0.99$) and 0.89 ($r^2 = 0.98$) for *hhyL*, 16S rRNA gene and IS respectively. The Perfecta SYBR Green Fast Mix (Quanta Biosciences®, Gaithersburg, Maryland, U.S.A.) was used for the qPCR, performed in a Rotor-Gene 6000 qPCR cycler (Corbett Life Science®, Concorde, New South Wales, Australia).

2.5. Pyrosequencing of bacterial 16S rRNA gene

Fully replicated ribotyping profiles were obtained by multiplex PCR-amplicon pyrosequencing, where each of the 12 DNA samples collected in the Netherlands was assigned to a unique barcoded primer set (Comeau et al., 2011). PCR amplification of the V6–V8 regions, library construction and multiplex 454 pyrosequencing reactions (GS FLX Plus, Roche) were performed at the Institut de Biologie Intégrative et des Systèmes (Université Laval, Québec, Canada), resulting in 94,431 raw sequences. The software mothur (Schloss et al., 2011) was utilized for sequence quality control and classification. Briefly, quality filtering was initiated using the pyrosequencing flow values. Sequences displaying errors in the barcode (rejected if >1 error) or primer (rejected if >2 errors) were then removed from the database comprising 400 bp average sequencing read length. Sequences were aligned using the Silva bacterial database (Pruesse et al., 2007) and 2914 chimeras detected using Uchime (Edgar et al., 2011) were removed from the dataset, resulting to 48,585 high-quality sequences. The database was de-multiplexed before randomly selecting 1021 sequences from each sample for classification of operational taxonomic units (OTU) at the 95% identity cut-off, rarefaction curves, microbial diversity and richness indexes analysis. Alignment and taxonomic assignment of the sequences was performed against the Silva bacterial database (Pruesse et al., 2007). Raw pyrosequencing reads have been deposited in the Sequence Read Archive of NCBI with the accession number SRP045104.

2.6. Statistical analysis

Statistical analyses were performed using the software R (R Development Core Team, 2008). Redundancy analysis (RDA) was computed using the package Vegan (Oksanen et al., 2012), according to the comprehensive procedure described by Borcard et al. (2011). RDA was considered to identify environmental variables influencing 16 rRNA gene profiles in soil and to highlight phyla whose relative abundance is related to elevated H_2 soil uptake activity. Soil variables (*e.g.* pH, carbon, nitrogen, H_2 uptake activity) were standardized by subtracting the average from the individual values and then dividing by the standard deviation. The Hellinger transformation was applied to the 16S OTU relative abundance dataset before computing the distance matrix to avoid undue relationships between explanatory variables and microbial community composition that could be caused by the heavy weighting of rare phylotypes (Legendre and Gallagher, 2001). The most parsimonious constrained model to explain the composition of microbial communities was obtained by forward selection of the environmental variables (Blanchet et al., 2008) and permutations ($n = 1000$) of the 16S data matrix were performed to verify the significance of the RDA. Pearson correlation analyses were conducted to identify environmental variables related to soil H_2 uptake activity as well as the co-linearity among variables. Single and multiple regression analyses were used to identify the best predictors for H_2 uptake activity in soil. Comparison of H_2 uptake activity and abundance of HOB between the land-use types was done by computing the Kruskal–Wallis analysis of variance on ranks using the Tukey *post-hoc* statistical test.

3. Results

3.1. Soil physicochemical properties and H_2 uptake activity

Soil encompassed a broad range of physicochemical properties, with the highest concentrations of phosphorus and potassium in the grassland and more carbon in forests (Table 1). Soil pH was positively correlated with phosphorus and potassium concentrations (Pearson, $p < 0.001$) and the same trend was observed between total carbon and nitrogen (Pearson, $p < 0.001$). Incubation of conditioned soil microcosms was necessary for the activation of HOB and the establishment of H_2 uptake activity. The activity increased by 47–119% during the first 24 h and remained at the same level on the second and the third day of the incubation period (Fig. 1). Grassland samples showed significantly less H_2 uptake activity than the forest samples (Kruskal–Wallis, $p = 0.016$). This dichotomy between grassland and forest samples reflected the contrasting physicochemical properties of soil. Indeed, H_2 soil uptake activity increased as a function of total carbon and nitrogen content (Pearson, $p < 0.02$), while pH, phosphorus and potassium concentrations displayed negative correlations with the H_2 uptake activity (Pearson, $p < 0.004$).

3.2. Microbial communities and HOB in soil

Pyrosequencing of bacterial 16S rRNA gene was undertaken to test whether microbial community structure can be used as predictor for soil uptake of atmospheric H_2. The sampling effort (*i.e.* 1021 sequences per sample) was sufficient to survey the relative abundance of the most abundant members comprising the communities. *Actinobacteria*, *Proteobacteria* and *Acidobacteria* dominated the soil microbial communities, representing on average 37, 35 and 14% of retrieved sequences, respectively (Fig. 2A). The relative abundance of *Actinobacteria* was indistinguishable between the grassland and forest soil samples (Kruskal–Wallis, $p = 0.36$). *Bacteriodetes* and *Firmicutes* were more abundant in grassland than forest soils (Kruskal–Wallis, $p = 0.016$). In contrast, the relative abundance of *Acidobacteria* and *Proteobacteria* was higher in forest than grassland soil (Kruskal–Wallis, $p = 0.016$). A RDA was performed to infer the relationship of 16S rRNA gene sequences with environmental variables. The most parsimonious model to explain the variation of bacterial 16S rRNA gene sequences included total carbon content and phosphorus concentration in soil (Fig. 3). The other variables were redundant with carbon and phosphorus content, and their inclusion increased the variance inflation factor unduly and they were therefore ignored for the analysis (Borcard et al., 2011). The first two canonical axes of the parsimonious RDA explained 67% of the total variance of 16S OTU relative abundance distribution ($p = 0.001$). Soil phosphorus concentration played an important role in the dispersion of the samples along the first axis, while carbon content differentiated the samples along the second. The forest and grassland soils were clearly separated along both axes (Fig. 3). Including H_2 soil uptake activity in the forward selection procedure did not improve the resolution of the RDA analysis. Indeed, replicated samples of mature spruce displaying 25% variation in H_2 oxidation rate (Fig. 1B) were characterized by highly similar ribotyping profiles (Fig. 3), while beech and grassland replicates exhibited more variable microbial community structures but only had 4 and 3% variation in H_2 uptake activity (Fig. 1A,D), respectively. This inconsistency between the bacterial ribotyping profile and *u* was also confirmed by single regression analyses that found that the relative abundance of bacterial phyla (*i.e. Acidobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes* or *Proteobacteria*) explained less than 42% of the total variation of measured H_2 oxidation activities. RDA analysis computed using 16S rRNA sequences classified at the class, order or family levels led to similar conclusions. Soil carbon, nitrogen and potassium content explained most variability in sequence distribution and including H_2 oxidation rate in the forward selection procedure did not improve the resolution of the RDA analyses (Table S2).

The lack of a relationship between the relative abundance of *Actinobacteria* and H_2 soil oxidation activity led us to ask whether only few bacteria in this phylum possess high affinity hydrogenase. To address this, we assessed the proportion of bacterial biomass representing HOB in soil microcosms by quantifying the number of *hhyL* and 16S rRNA genes by qPCR, normalized using an internal standard. Microcosm *hhyL* gene abundances were between 5.6×10^7 and 4.9×10^8 $g^{-1}_{(soil\text{-}dw)}$ and 16S rRNA genes were between 2.0×10^{11} and 2.5×10^{12} copies $g^{-1}_{(soil\text{-}dw)}$. The relative abundance of HOB was between 0.02 and 0.1%, assessed by the number of *hhyL* genes normalized to the number of 16S rRNA genes, indicating that HOB are members of the rare biosphere (Fig. 2B). This is a rough estimate because some microbes harbor more than one 16S rRNA gene operon on their chromosome and additional bias is introduced by the coverage and specificity of the oligonucleotides utilized in both qPCR assays. The relative abundance of HOB was significantly lower in grassland than forest soil samples (Kruskal–Wallis, $p = 0.008$), but this variable was not a suitable predictor for *u* in a single linear regression analysis ($R^2 = 0.04$, $p = 0.54$).

3.3. Identification of explanatory variables for the soil uptake of atmospheric H_2

Soil carbon content was the best predictor for *u* (Table 2, eq. (1)), explaining 66% of the total variation of the activity (Fig. 4). Based on the fact that microorganisms are involved in the activity, we performed a stepwise forward selection of molecular predictors with the goal of developing multiple regression models with more explanatory power. The relative abundance of *Actinobacteria* and *hhyL*/16S rRNA gene qPCR ratio were selected as secondary predictors because most of the HOB identified to date belong to the *Actinobacteria* phylum and because the ratio of the *hhyL* to 16S rRNA genes represents the enrichment factor of this functional group in soil and previous investigations showed that absolute *hhyL* gene abundance alone was not a suitable proxy for H_2 oxidation activity in soil (Constant et al., 2011b). Inclusion of the *Actinobacteria* relative abundance did not improve the prediction based

Table 1
Physicochemical properties of soil, number of *hhyL* and 16S rRNA genes and diversity of microbial communities based on 16S rRNA gene (OTU 95% cut-off level) from the first soil survey. Average values (n = 3) are represented with standard deviations (in parenthesis).

Sites	C (%)	N (%)	P (mg kg^{-1})	K (mg kg^{-1})	pH	*hhyL* gene (copies $g^{-1}_{(dw)}$)	16S rRNA gene (copies $g^{-1}_{(dw)}$)	Shannon (H′)
Beech	31 (7.4)	1.4 (0.3)	380 (27)	298 (15)	5.3 (0.0)	$2.3 (0.3) \times 10^8$	$3.6 (0.6) \times 10^{11}$	6.2 (0.1)
Spruce (mature)	44 (1.8)	1.6 (0.2)	319 (32)	175 (19)	2.8 (0.2)	$1.0 (0.8) \times 10^8$	$2.4 (0.2) \times 10^{11}$	6.0 (0.1)
Spruce (young)	13 (4.3)	0.6 (0.2)	184 (6)	167 (74)	3.2 (0.1)	$1.3 (0.4) \times 10^8$	$2.5 (0.5) \times 10^{11}$	6.2 (0.1)
Grassland	8.6 (0.2)	0.7 (0.0)	949 (25)	2218 (48)	5.2 (0.0)	$1.9 (0.3) \times 10^8$	$8.2 (1.5) \times 10^{11}$	6.5 (0.0)

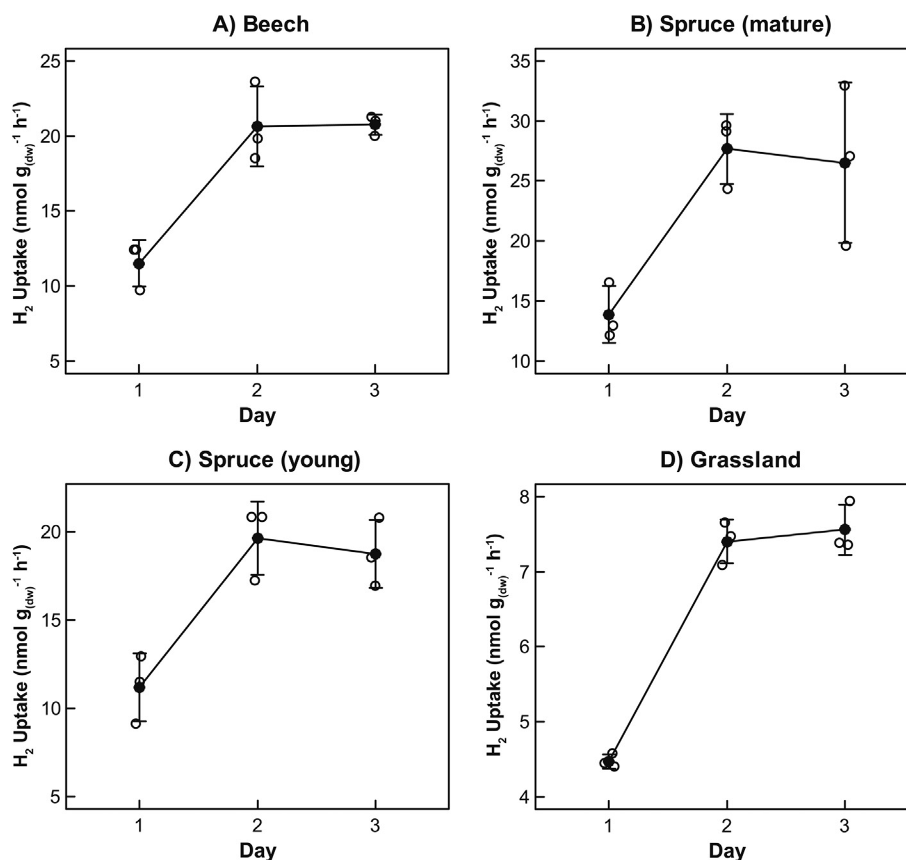


Fig. 1. H_2 soil uptake activity measured in (A) beech forest, (B) mature spruce forest, (C) young spruce forest and (D) grassland soil microcosms. The mean values and standard deviation of the H_2 soil uptake activity derived from the biologically independent triplicates (empty circles; ○) are represented by the filled circles (●).

on carbon content (Table S3). A multiple regression parameterized with total carbon and the relative abundance of HOB resulted in minor improvement in model prediction (Table 2, eq. (2)). The best model was obtained by accounting for the interaction between soil carbon content and the relative abundance of HOB in soil (Table 2, eq. (3)), explaining 92% of the total variation of the H_2 oxidation activity (Fig. 4). Attempts to improve the model were made by considering the relative abundance of 16S rRNA sequences classified at different taxonomic levels (*i.e.* class, order or family). Even though the relative abundance of some taxa showed significant correlation with H_2 oxidation rate in soil, they did not improve the predictions based on carbon content and HOB (Table S3).

3.4. Validation of identified explanatory variables with additional samples

Additional soil samples were collected in deciduous forests, maize monocultures, larch plantations and grasslands to validate the explanatory variables identified in the first soil survey. Soil microcosms were investigated for H_2 uptake activity, the relative abundance of HOB and physicochemical characteristics (Table S1). H_2 oxidation activity ranged from 1.2 nmol g^{-1} h^{-1} in a larch monoculture (station WEN-M3) to 26 nmol g^{-1} h^{-1} in a deciduous forest (station Harvard-F2). Comparison of u measured in deciduous forests, agricultural fields, larch monocultures and grasslands revealed significantly higher activity in deciduous forests than other land-use types (Fig. 5). The relative abundance of HOB estimated by qPCR supported the previous finding that HOB are part of the rare biosphere, representing 0.04–1.0% of the bacterial biomass (Table S1). The relative abundance of HOB was not influenced by land-use type (Kruskal–Wallis, $p = 0.34$). Regression analyses were performed to determine the relationship between total carbon content and the relative abundance of HOB with H_2 uptake activity. Single linear regression parameterized with total carbon content explained 49% of the variability in u (Table 2, eq. (4)). Model residuals were not randomly distributed and followed a pattern defined by land-use type (Fig. 6A). Predicted u were systematically overestimated by factor of 4.2 ± 2.2 in maize monocultures, larch plantations and grasslands, while larger variations were observed for soil samples collected in deciduous forests. For deciduous forest samples, residuals were scattered as a function of soil texture class with overestimation of u by factor of 2.8 ± 1.3 in loamy soils and underestimations by factor of 1.2 ± 0.14 in silty soils. The influence of soil texture class on u prediction in deciduous forests was less prominent in the multiple regression parameterized with carbon and relative abundance of HOB (Fig. 6B). This model explained 87% of the variability in u (Table 2, eq. (5)) and addition of soil texture variables did not improve predictive power ($R^2 = 0.87$, $p = 0.001$). Indeed, assessment of the relative importance of all measured variables unveiled that total carbon content and the relative abundance of HOB were the best predictors for u (Fig. S1). In contrast to the first soil survey, consideration of the interaction between HOB and carbon in soil resulted to modest reduction of model residuals (Table 2, eqs. (5)–(6)).

3.5. Predictive model for H_2 uptake activity in soil

H_2 oxidation activity, abundance of HOB and total carbon content datasets obtained in the two independent soil surveys were combined and utilized to derive three predictive models for u

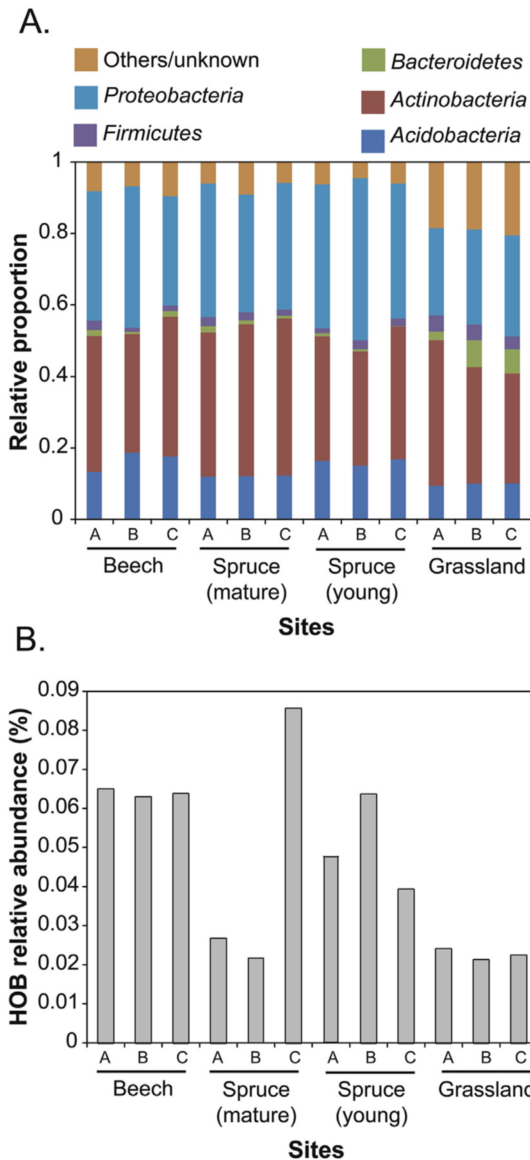


Fig. 2. Microbial community structure in soil microcosms after 3 days of incubation. (A) Distribution of bacterial 16S rRNA gene sequences classified at the phylum taxonomic level across the four land-use types. (B) Relative abundance of HOB inferred by qPCR (abundance of *hhyL* to that of 16S rRNA gene detected in soil microcosms). The results of the triplicate microcosms are presented.

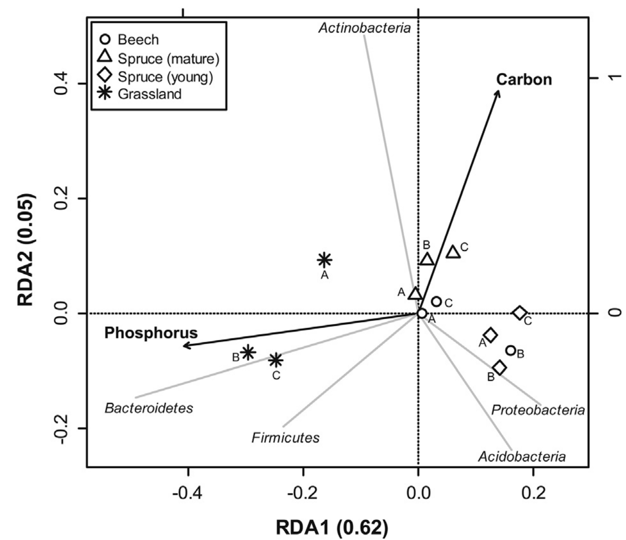


Fig. 3. Parsimonious RDA triplot of Hellinger-transformed relative frequency matrix of bacterial 16S rRNA gene sequences classified at the phylum level explained by soil total carbon and phosphorus contents. The position of replicated soil microcosms reflects their bacterial 16S rRNA gene profile.

(Table 2, eqs. (7)–(9)). The results indicate that better fits to the data were obtained using the multiple regression analysis but interaction between carbon content and HOB exerted a negligible impact on model residuals (Table 2, eqs. (8)–(9)). We recommend the first multiple regression model for prediction of u in soil (Table 2, eq. (8)). However, the residuals of deciduous and spruce forest samples exhibited more variability than the other land-use types, suggesting a potential limitation of the model performance in forest ecosystems (Fig. S1). Addition of total soil nitrogen content in forward selection of explanatory variables did not improve prediction of u in soil (data not shown).

4. Discussion

Progress in sequencing technologies has changed perspectives of biogeochemistry. The descriptive analysis of microbial community structure has been replaced with genetic profiles for the purpose of predicting process rates in the environment and detecting chemical transformations that are difficult to observe using analytical chemistry (Zak et al., 2006). Furthermore, the growing database of genetic sequences has led to the emergence of some level of coherence between the taxonomic ranks of microorganisms and their ecological niche to emerge (Philippot et al., 2010). These relationships were observed for higher taxonomic ranks than microbial species (*e.g.* phylum level), leading to the suggestion that ribotyping profiles obtained by high-throughput sequencing techniques could be used to characterize the nutrient status, microbial metabolism or even biogeochemical processes taking place in a given environment (Langille et al., 2013). The classical example of such an approach is the relationship between the relative abundance of certain phyla and CO_2 respiration rates in soil. In a combination of meta-analysis and laboratory incubation experiments, *β-Proteobacteria*, *Bacteroidetes* and soil respiration were correlated, while *Acidobacteria* showed the inverse trend (Fierer et al., 2007). This observation led to the assumption that abundant *β-Proteobacteria* and *Bacteroidetes* are indicative of copiotrophic microbial communities (r-strategists) while abundant *Acidobacteria* are indicative of oligotrophic communities (k-strategists).

Considering the fact that atmospheric H_2 supplies maintenance and survival energy to HOB (Constant et al., 2011b), we hypothesized that the relative abundance of k-strategists or bacterial taxonomic groups associated to oligotrophic ecological niches could be used as a proxy for H_2 uptake activity. However, it appears that neither the relative abundance of bacterial lineages associated with oligotrophic communities nor the abundance of *Actinobacteria* is suitable predictor for u in soil. This observation supports the notion that HOB are ubiquitously distributed in the environment, with the concomitant occurrence and activity of this functional group reported in diverse ecosystems ranging from oligotrophic arid deserts to copiotrophic temperate forests and subactic peatlands (Constant et al., 2011b). The qPCR assays targeting *hhyL* and 16S rRNA genes revealed that HOB are members of the rare biosphere, and represent less than 1% of total bacterial biomass in soil. The relatively low resolution of the ribotyping profiles obtained in this study provided an incomplete picture of the microbial assemblage composition and precluded recovery of the rare

Table 2
Single and multiple regressions derived with observations made in the first and the second soil survey showing the relationship of *u* with total carbon content and the relative abundance of HOB. Predictive models were calculated after combining the datasets of both surveys.

Equations		R^2 (p-value)	Residual standard error (ε)
Linear regression derived from the first soil survey (n = 12)			
1	$u = 0.42(\pm 0.09)C + 8.31(\pm 2.64) + \varepsilon$	0.66 (0.001)	4.7
2	$u = 0.42(\pm 0.10)C - 1007(\pm 6974)\text{HOB} + 8.67(\pm 3.73) + \varepsilon$	0.67 (0.007)	5.0
3	$u = 0.90(\pm 0.10)C + 36,292(\pm 7939)\text{HOB} - 1249(\pm 238)\text{C} \times \text{HOB} - 4.95(\pm 3.21) + \varepsilon$	0.92 (<0.0001)	2.5
Linear regression derived from the second soil survey (n = 14)			
4	$u = 0.62(\pm 0.18)C + 3.52(\pm 2.27) + \varepsilon$	0.49 (0.005)	6.4
5	$u = 0.32(\pm 0.11)C + 1814(\pm 329)\text{HOB} + 1.68(\pm 1.27) + \varepsilon$	0.87 (<0.0001)	3.5
6	$u = 0.29(\pm 0.23)C + 1591(\pm 1578)\text{HOB} - 14(\pm 96)\text{C} \times \text{HOB} + 1.96(\pm 2.36) + \varepsilon$	0.87 (0.0001)	3.6
Predictive models derived from both soil surveys (n = 26)			
7	$u = 0.53(\pm 0.08)C + 4.97(\pm 1.64) + \varepsilon$	0.66 (<0.0001)	5.7
8	$u = 0.52(\pm 0.07)C + 1190(\pm 371)\text{HOB} + 3.27(\pm 1.49) + \varepsilon$	0.76 (<0.0001)	4.8
9	$u = 0.59(\pm 0.09)C + 2710(\pm 1533)\text{HOB} - 85(\pm 83)\text{C} \times \text{HOB} + 1.95(\pm 1.96) + \varepsilon$	0.77 (<0.0001)	4.8

biosphere, impairing identification of the rare phylotypes responsible for H_2 uptake activity in soil (Bartram et al., 2011). More sequencing efforts, resulting to a better coverage of individual members of the microbial community, would be necessary to investigate if the relative abundance of certain rare phylotypes can be used as a predictor of *u* in soil. However, the high affinity hydrogenase is unevenly distributed in *Actinobacteria* and to a lesser extent in *Acidobacteria*, *Chloroflexi*, and *Proteobacteria* (Constant et al., 2011b), which clearly limits the exploitation of bacterial ribotyping profiles to predict *u* in soil.

The soil uptake of atmospheric H_2 is controlled by two main parameters: H_2 diffusion and metabolic activity of HOB. While the diffusion term of the activity is relatively well constrained by diffusion models, the environmental factors influencing the distribution and metabolic activity of HOB are far from being elucidated. For instance, elevated soil carbon content was associated with high H_2 deposition velocities in boreal forests (Rahn et al., 2002; Lallo et al., 2008), while no significant relationship was observed in sub-tropical regions (Conrad and Seiler, 1985). These contrasting results might be explained by the concomitant diffusion limitation of the H_2 uptake activity in investigated soils and the ecophysiology of HOB. A robust analysis of the factors influencing microbial H_2 uptake activity in soil requires controlled conditions to limit the impact of sample-specific diffusion limitations. In our methodological approach we size fractionated soil particles, adjusted soil water content to 20% whc and utilized an adequate ratio of soil to headspace volumes (where the activity is directly proportional to soil weight) in controlled soil microcosms, which enabled comparison of *u* in samples collected in different land-use types. Higher H_2 oxidation activity in forests than in maize monocultures, larch plantations and grasslands provided experimental evidence for higher activity of HOB expected in temperate forests (Ehhalt and Rohrer, 2009). As variability of HOB abundance was not explained by land-use type, it is likely that stimulation of hydrogenase activity rather than increasing HOB biomass triggered the high H_2 uptake activities observed in forest soils. In contrast to deciduous and spruce forests, larch plantations showed low H_2 oxidation potential, with values indistinguishable from grasslands and maize monocultures. This can be explained by the fact that

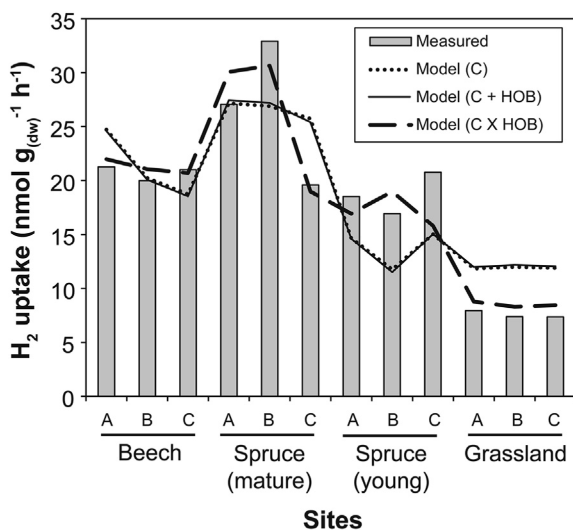


Fig. 4. H_2 soil uptake activity as measured using the gas chromatographic assay (gray bars), modeled using a single regression model with total carbon content as predictor, and modeled with a multiple regression combining total carbon content and the relative abundance of HOB (*hhyL*/16S ratio) as predictors. The expressions (C + HOB) and (C × HOB) refer to regression parameterization not considering and considering interactions between both variables, respectively. Analyses were derived from the dataset of the first soil survey (n = 12).

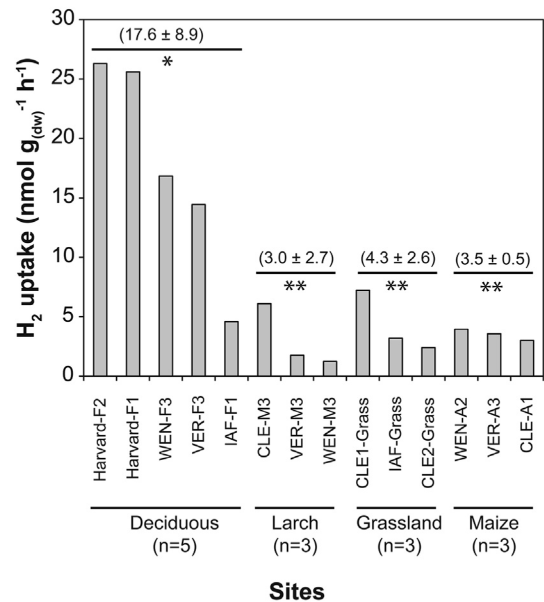


Fig. 5. H_2 soil uptake activity measured in soil samples collected in deciduous forests, larch plantations, grasslands and maize monocultures. Average and standard deviation were calculated for each land-use type and different symbols (* and **) denote significant difference between the mean activities as a function of land-use type (ANOVA, $p < 0.05$).

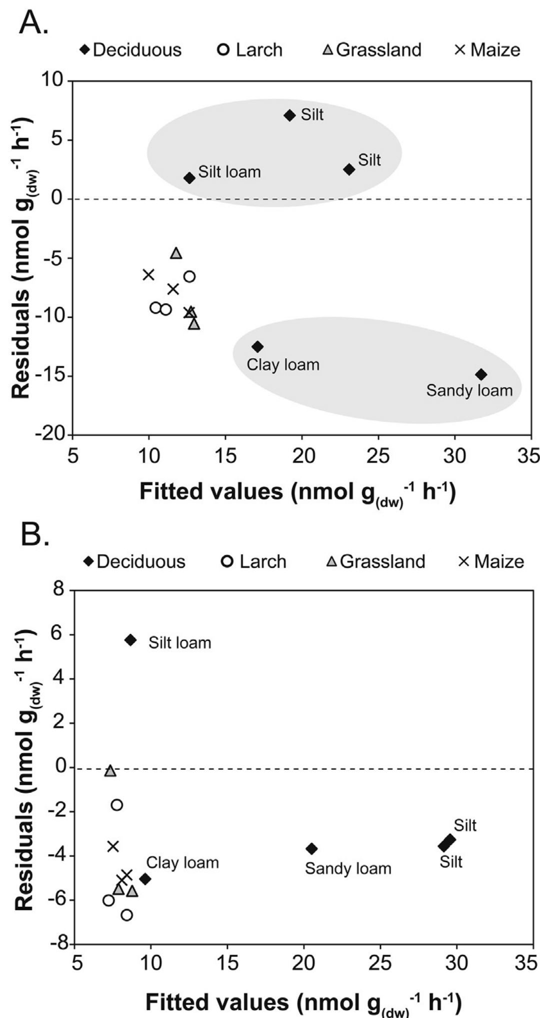


Fig. 6. Residual plot for H_2 soil uptake activity to assess the assumption of linear regression with (A) soil total carbon content alone or (B) total carbon content and the relative abundance of HOB as prediction variables. Analyses were derived from the dataset of the second soil survey (n = 14). The gray areas highlight the overestimation of u in loamy deciduous forest soils and underestimation of u in silty deciduous forest soils (residual value = experimental − fitted H_2 uptake rate).

surveyed larch plantations were seeded on areas that had been used for agriculture 15 years ago and were characterized by lower soil carbon and nitrogen contents than surveyed deciduous and spruce forests (Table S1). Total soil carbon content was the best performing variable in our database for explaining variance in u, but the predictions were influenced by soil texture in the model parameterized with this single variable in deciduous forest soil samples (Fig. 6A). According to model residuals analysis, u was overestimated in loamy soils, but underestimated in silty soils of deciduous forest. The multiple regression model parameterized with total carbon and the relative abundance of HOB significantly improved model performance (Table 2). This can be interpreted as pointing to an influence of soil structure on the stimulation of HOB activity. Although the exact mechanisms behind the relationship between soil carbon content and u are unknown, there is no doubt that carbon sources must be available to sustain the growth of HOB mostly displaying heterotrophic and to a lesser extend facultative chemolithotrophic modes of metabolism. Instead of supporting autotrophic growth, the energy yield of the oxidation of atmospheric H_2 likely supports survival and mixotrophic metabolism of bacteria (Conrad, 1999; Berney and Cook, 2010; Constant et al., 2010, 2011b; Berney et al., 2014; Greening et al., 2015). Besides the HOB, soil carbon is utilized by a broad variety of microorganisms and thus shapes the whole structure of microbial communities (de Vries et al., 2012). Microbial interactions such as competition for carbon and other nutrients may trigger activation of high affinity hydrogenase in HOB, resulting in another potential impact of soil carbon on u in soil.

The predictive multiple regression models derived in this study (Table 2, eqs. (7)–(9)) are a significant development for the understanding of H_2 biogeochemistry. This is the first attempt to include the relative abundance of HOB for prediction of u in soil. However, the residual analysis highlighted some potential limitations for the application of the predictive models of u for forest ecosystems. Furthermore, there are probably other variables than those we measured to consider before parameterizing u using the multiple regressions presented in Table 2 in atmospheric H_2 distribution models. We propose three research directions to identify those parameters influencing HOB ecophysiology and improve u predictions. Firstly, the distribution of HOB among various soil particle size fractions must be addressed. The H_2 oxidation rates reported in this study were measured from homogenized soil microcosms (2 mm sieve), and the heterogeneous distribution of HOB in particular soil fractions characterized by different nutrients and trace metals contents would influence model performance for bulk soil samples. Secondly, the models derived in this study need to be challenged with soil samples originating from other globally important land-use types such as deserts, peatlands and wetlands. These ecosystems act as important sinks for atmospheric H_2 and are characterized by soil encompassing a broad range in physicochemical properties and populations of HOB (Constant et al., 2011b). Finally, it would be of significant interest to parameterize predictive models of u incorporating *hhyL* expression dynamics in soil. High affinity hydrogenase gene expression is not constitutive in HOB. The enzyme is activated in dormant spores of *Streptomyces* (Constant et al., 2008, 2010) as well as starved *Rhodococcus* (Meredith et al., 2013) and *Mycobacterium* (King, 2003; Berney and Cook, 2010) cells, highlighting inherent limitation of the ratio of *hhyL* to 16S rRNA gene abundance to predict u in soil. Such a gene expression approach was utilized to predict methane emissions, where the expression profile of the *mcr*A gene encoding the methyl coenzyme M reductase in methanogenic archaea showed some relation to the methane production rate in peat slurries (Freitag and Prosser, 2009). However, hydrogenase maturation and assembly in HOB requires several auxiliary proteins and any influence of environmental factors on posttranslational machinery might impair prediction of u based on *hhyL* gene expression. This was observed in the case of the nitrous oxide reductase gene (*nosZ*) catalyzing the reduction of nitrous oxide to nitrogen in denitrifying organisms. It appeared that soil pH influenced the activity of accessory proteins involved in NosZ maturation, resulting in transcription of the *nosZ* gene in the absence of detectable nitrous oxide reduction in acidic soil microcosms (Liu et al., 2014). Analysis of *hhyL* transcription level in soil is currently in progress in the author's laboratory to relate the expression profile of the gene with H_2 uptake activity.

In conclusion, molecular biogeochemistry holds great promise to assess the fate and distribution of biogeochemical processes in the environment. This work shows the potential of using a molecular marker combined with physicochemical parameters to predict u in soil. The strength of molecular-based models is that they allow for the parameterization of a microbial physiological response to environmental conditions, which can lead to predictions of process rates and interactions between microorganisms involved in element cycles. Oxidation of atmospheric H_2 by soil HOB can now be considered as a new example of a large-scale process on Earth sustained by the rare biosphere. Even though

they represent less than 1% of the bacterial biomass in soil, HOB mitigate global H_2 emissions and may help maintain the atmospheric H_2 burden at steady-state, which justifies further investigations into their distribution and activity.

Acknowledgments

LH and IL are grateful to the Foundation Universitaire Armand-Frappier INRS for their M.Sc. scholarships. This work was supported by a Natural Sciences and Engineering Research Council of Canada Discovery grant (418252-2012) to PC.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.02.030.

References

Bartram, A.K., Lynch, M.D.J., Stearns, J.C., Moreno-Hagelsieb, G., Neufeld, J.D., 2011. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. Applied and Environmental Microbiology 77, 3846–3852.
Berney, M., Cook, G.M., 2010. Unique flexibility in energy metabolism allows mycobacteria to combat starvation and hypoxia. PLoS ONE 5, e8614.
Berney, M., Greening, C., Hards, K., Collins, D., Cook, G.M., 2014. Three different NiFe hydrogenases confer metabolic flexibility in the obligate aerobe *Mycobacterium smegmatis*. Environmental Microbiology 16, 318–330.
Blanchet, F.G., Legendre, P., Borcard, D., 2008. Forward selection of explanatory variables. Ecology 89, 2623–2632.
Borcard, D., Gillet, F., Legendre, P., 2011. Numerical Ecology with R. Springer, New York.
Comeau, A.M., Li, W.K.W., Tremblay, J.-É., Carmack, E.C., Lovejoy, C., 2011. Arctic Ocean microbial community structure before and after the 2007 Record Sea Ice Minimum. PLoS ONE 6, e27492.
Conrad, R., 1999. Soil microorganisms oxidizing atmospheric trace gases (CH_4, CO, H_2, NO). Indian Journal of Microbiology 39, 193–203.
Conrad, R., Seiler, W., 1985. Influence of temperature, moisture, and organic carbon on the flux of H_2 and CO between soil and atmosphere: field studies in subtropical regions. Journal of Geophysical Research: Atmospheres 90, 5699–5709.
Constant, P., Chowdhury, S.P., Hesse, L., Conrad, R., 2011a. Co-localization of atmospheric H_2 oxidation activity and high affinity H_2-oxidizing bacteria in non-axenic soil and sterile soil amended with *Streptomyces* sp. PCB7. Soil Biology and Biochemistry 43, 1888–1893.
Constant, P., Chowdhury, S.P., Hesse, L., Pratscher, J., Conrad, R., 2011b. Genome data mining and soil survey for the novel Group 5 [NiFe]-hydrogenase to explore the diversity and ecological importance of presumptive high affinity H_2-oxidizing bacteria. Applied and Environmental Microbiology 77, 6027–6035.
Constant, P., Chowdhury, S.P., Pratscher, J., Conrad, R., 2010. Streptomycetes contributing to atmospheric molecular hydrogen soil uptake are widespread and encode a putative high-affinity [NiFe]-hydrogenase. Environmental Microbiology 12, 821–829.
Constant, P., Poissant, L., Villemur, R., 2008. Isolation of *Streptomyces* sp. PCB7, the first microorganism demonstrating high-affinity uptake of tropospheric H_2. ISME Journal 2, 1066–1076.
Constant, P., Poissant, L., Villemur, R., 2009. Tropospheric H_2 budget and the response of its soil uptake under the changing environment. Science of the Total Environment 407, 1809–1823.
de Vries, F.T., Manning, P., Tallowin, J.R.B., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., Hobbs, P.J., Quirk, H., Shipley, B., Cornelissen, J.H.C., Kattge, J., Bardgett, R.D., 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology Letters 15, 1230–1239.
DeCoste, N.J., Gadkar, V.J., Filion, M., 2011. Relative and absolute quantitative real-time PCR-based quantifications of *hcnC* and *phlD* gene transcripts in natural soil spiked with *Pseudomonas* sp. strain LBUM300. Applied and Environmental Microbiology 77, 41–47.
Deer, D.M., Lampel, K.A., González-Escalona, N., 2010. A versatile internal control for use as DNA in real-time PCR and as RNA in real-time reverse transcription PCR assays. Letters in Applied Microbiology 50, 366–372.
Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200.
Ehhalt, D.H., Rohrer, F., 2009. The tropospheric cycle of H_2: a critical review. Tellus B 61, 500–535.
Ehhalt, D.H., Rohrer, F., 2011. The dependence of soil H_2 uptake on temperature and moisture: a reanalysis of laboratory data. Tellus B 63, 1040–1051.
Ehhalt, D.H., Rohrer, F., 2013. Deposition velocity of H_2: a new algorithm for its dependence on soil moisture and temperature. Tellus B 65, 19904.
Elghamry, W., Elashkar, M., 1962. Simplified textural classification triangles. Soil Science Society of America Journal 26, 612–613.
Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. Ecology 88, 1354–1364.
Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71, 4117–4120.
Freitag, T.E., Prosser, J.I., 2009. Correlation of methane production and functional gene transcriptional activity in a peat soil. Applied and Environmental Microbiology 75, 6679–6687.
Gödde, M., Meuser, K., Conrad, R., 2000. Hydrogen consumption and carbon monoxide production in soils with different properties. Biology and Fertility of Soils 32, 129–134.
Greening, C., Berney, M., Hards, K., Cook, G.M., Conrad, R., 2014. A soil actinobacterium scavenges atmospheric H_2 using two membrane-associated, oxygen-dependent [NiFe] hydrogenases. Proceedings of the National Academy of Sciences of the United States of America 111, 4257–4261.
Greening, C., Constant, P., Hards, K., Morales, S.E., Oakeshott, J.G., Russell, R.J., Taylor, M.C., Berney, M., Conrad, R., Cook, G.M., 2015. Atmospheric hydrogen scavenging: from enzymes to ecosystems. Applied and Environmental Microbiology 81, 1190–1199.
King, G.M., 2003. Uptake of carbon monoxide and hydrogen at environmentally relevant concentrations by mycobacteria. Applied and Environmental Microbiology 69, 7266–7272.
Lallo, M., Aalto, T., Laurila, T., Hatakka, J., 2008. Seasonal variations in hydrogen deposition to boreal forest soil in southern Finland. Geophysical Research Letters 35, L04402.
Langille, M.G., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Thurber, R.L.V., Knight, R., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature Biotechnology 31, 814–821.
Legendre, P., Gallagher, E., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271–280.
Liu, B., Frostegård, Å., Bakken, L.R., 2014. Impaired reduction of N_2O to N_2 in acid soils is due to a posttranscriptional interference with the expression of *nosZ*. mBio 5, e01383-14.
Meredith, L.K., Commane, R., Munger, J.W., Dunn, A., Tang, J., Wofsy, S.C., Prinn, R.G., 2014. Ecosystem fluxes of hydrogen: a comparison of flux-gradient methods. Atmospheric Measurement Techniques 7, 2879–2928.
Meredith, L.K., Rao, D., Bosak, T., Klepac-Ceraj, V., Tada, K.R., Hansel, C.M., Ono, S., Prinn, R.G., 2013. Consumption of atmospheric hydrogen during the life cycle of soil-dwelling actinobacteria. Environmental Microbiology Reports 6, 226–238.
Novelli, P.C., Lang, P.M., Masarie, K.A., Hurst, D.F., Myers, R., Elkins, J.W., 1999. Molecular hydrogen in the troposphere: global distribution and budget. Journal of Geophysical Research 104, 30427–30444.
Oksanen, J., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2012. Vegan: Community Ecology Package. R Package Version 2.0-4.
Pendall, E., Schwendenmann, L., Rahn, T., Miller, J.B., Tans, P.P., White, J.W.C., 2010. Land use and season affect fluxes of CO_2, CH_4, CO, N_2O, H_2 and isotopic source signatures in Panama: evidence from nocturnal boundary layer profiles. Global Change Biology 16, 2721–2736.
Philippot, L., Andersson, S.G., Battin, T.J., Prosser, J.I., Schimel, J.P., Whitman, W.B., Hallin, S., 2010. The ecological coherence of high bacterial taxonomic ranks. Nature Reviews Microbiology 8, 523–529.
Pieterse, G., Krol, M.C., Batenburg, A.M., Brenninkmeijer, C.A.M., Popa, M.E., O'Doherty, S., Grant, A., Steele, L.P., Krummel, P.B., Langenfelds, R.L., Wang, H.J., Vermeulen, A.T., Schmidt, M., Yver, C., Jordan, A., Engel, A., Fisher, R.E., Lowry, D., Nisbet, E.G., Reimann, S., Vollmer, M.K., Steinbacher, M., Hammer, S., Forster, G., Sturges, W.T., Röckmann, T., 2013. Reassessing the variability in atmospheric H_2 using the two-way nested TM5 model. Journal of Geophysical Research: Atmospheres 118, 3764–3780.
Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., Glöckner, F.O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Research 35, 7188–7196.
Rahn, T., Eiler, J.M., Kitchen, N., Fessenden, J.E., Randerson, J.T., 2002. Concentration and δD of molecular hydrogen in boreal forests: ecosystem-scale systematics of atmospheric H_2. Geophysical Research Letters 29, 1888.
Schloss, P.D., Gevers, D., Westcott, S.L., 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. PLoS ONE 6, e27310.
Smith-Downey, N.V., Randerson, J.T., Eiler, J.M., 2008. Molecular hydrogen uptake by soils in forest, desert, and marsh ecosystems in California. Journal of Geophysical Research 113, G03037.
R Development Core, Team, 2008. R: a language and environment for statistical computing. In: Computing, R.F.f.S. (Ed.). Vienna, Austria.
Yonemura, S., Yokozawa, M., Kawashima, S., Tsuruta, H., 2000. Model analysis of the influence of gas diffusivity in soil on CO and H_2 uptake. Tellus B 52, 919–933.
Zak, D.R., Blackwood, C.B., Waldrop, M.P., 2006. A molecular dawn for biogeochemistry. Trends in Ecology and Evolution 21, 288–295.