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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

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ABSTRACT

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.

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.

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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