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Short Communication

Meta-omics survey of [NiFe]-hydrogenase genes fails to capture drastic variations in H_2-oxidation activity measured in three soils exposed to H_2

Mondher Khdhiri[a], Sarah Piché-Choquette[a], Julien Tremblay[b], Susannah G. Tringe[c], Philippe Constant[a,*]

[a] Institut National de la Recherche Scientifique-Institut Armand-Frappier, 531 boulevard des Prairies, Laval, Québec, H7V 1B7, Canada
[b] National Research Council Canada, 6100, avenue Royalmount, Montréal, Québec, H4P 2R2, Canada
[c] DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA, 94598, USA

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ABSTRACT

Inferences on soil biogeochemical processes based on metagenomic profiles is a challenging task due to enormous diversity of soil microbes and the fragile linkage between gene abundance and functioning. Here we used the biological sink of H_2 as a case study to test the hypothesis that [NiFe]-hydrogenase gene distribution and expression profiles explain variations in H_2 oxidation rate measured in soil collected in poplar monoculture, larch plantation and farmland. Shotgun metagenomic and metatranscriptomic analyses of soil samples exposed to elevated or low H_2 concentration led to the identification of 45 genes encoding the large subunit of [NiFe]-hydrogenases belonging to 8 distinct phyla. Our results indicate that despite significant sequencing effort, retrieved hydrogenase sequences are not in themselves adequate surrogates of H_2 oxidation activity in these soils. In fact, land-use exerted a greater influence than H_2 exposure on both hydrogenase gene distribution and expression though expression of certain genes responded to H_2. We argue that approaches relying on PCR/RT-PCR amplicon sequencing or quantification combined with physicochemical parameters are currently the best option to infer the activity of H_2-oxidizing bacteria and probably other specialist functional guilds with similar population size in soil.

Microbial community profiles are increasingly used to refine biogeochemical process models relying on abiotic factors and first-order kinetics governing reaction rates (Todd-Brown et al., 2012; Wieder et al., 2013; Powell et al., 2015; Pérez-Valera et al., 2015). Nevertheless, the appeal of this approach remains controversial. For instance, modeling diverse C and N process rates using functional or 16S rRNA genes quantification or profiling did not improve every single model previously based on geochemical factors (Graham et al. 2014, 2016; Reed et al., 2014). Implementation of reliable genomic proxies for elemental cycling in the environment must consider a number of factors including decoupling between gene expression and enzyme activity, occurrence of microbial dormancy, microbe ecophysiology and resolution of genomic techniques (Bailey et al., 2018).

In this study, we investigate the application of omics data into biogeochemical models using molecular hydrogen (H_2) as a case study. Aerated upland soils are the most important H_2 sinks where high-affinity H_2-oxidizing bacteria (HOB) are responsible for 70% of global losses of tropospheric H_2 (Pieterse et al., 2013) and low-affinity HOB scavenge H_2 produced by plant root nodules and fermentation reaction taking place in waterlogged niches, preventing its diffusion to the atmosphere (Schuler and Conrad, 1991). H_2 oxidation activity in aerobic HOB is conferred by [NiFe]-hydrogenases classified into five distinct functional groups according to their physiological role and cellular localization. Briefly, group 1 includes membrane-bound hydrogenases supplying electrons to the respiratory chain; group 2 are either H_2 sensors regulating the transcription of hydrogenases operons or recyclers of H_2 by-products of nitrogen fixation and supporters of aerobic hydrogenotrophic growth and survival (especially under carbon and oxygen limiting conditions); group 3 are used to regenerate reducing equivalents in the cells (*e.g.* NAD, F-420); group 4 are either energy converting enzymes that ensure the generation of proton motive force or hydrogenases that recycle the reducing equivalents from fermentative processes through H_2 production (Vignais and Billoud, 2007; Greening et al., 2016) and lastly, group 5 (or group 1h) encompasses high-affinity hydrogenases used for mixotrophic growth and survival (Constant et al., 2011). This classification scheme originating from the pioneering work of Vignais and Colbeau (2004) examining physiological role has been revisited through the use of sequence similarity

* Corresponding author. INRS-Institut Armand-Frappier, 531 boulevard des Prairies, Laval, Québec, H7V 1B7, Canada.
E-mail address: Philippe.Constant@iaf.inrs.ca (P. Constant).

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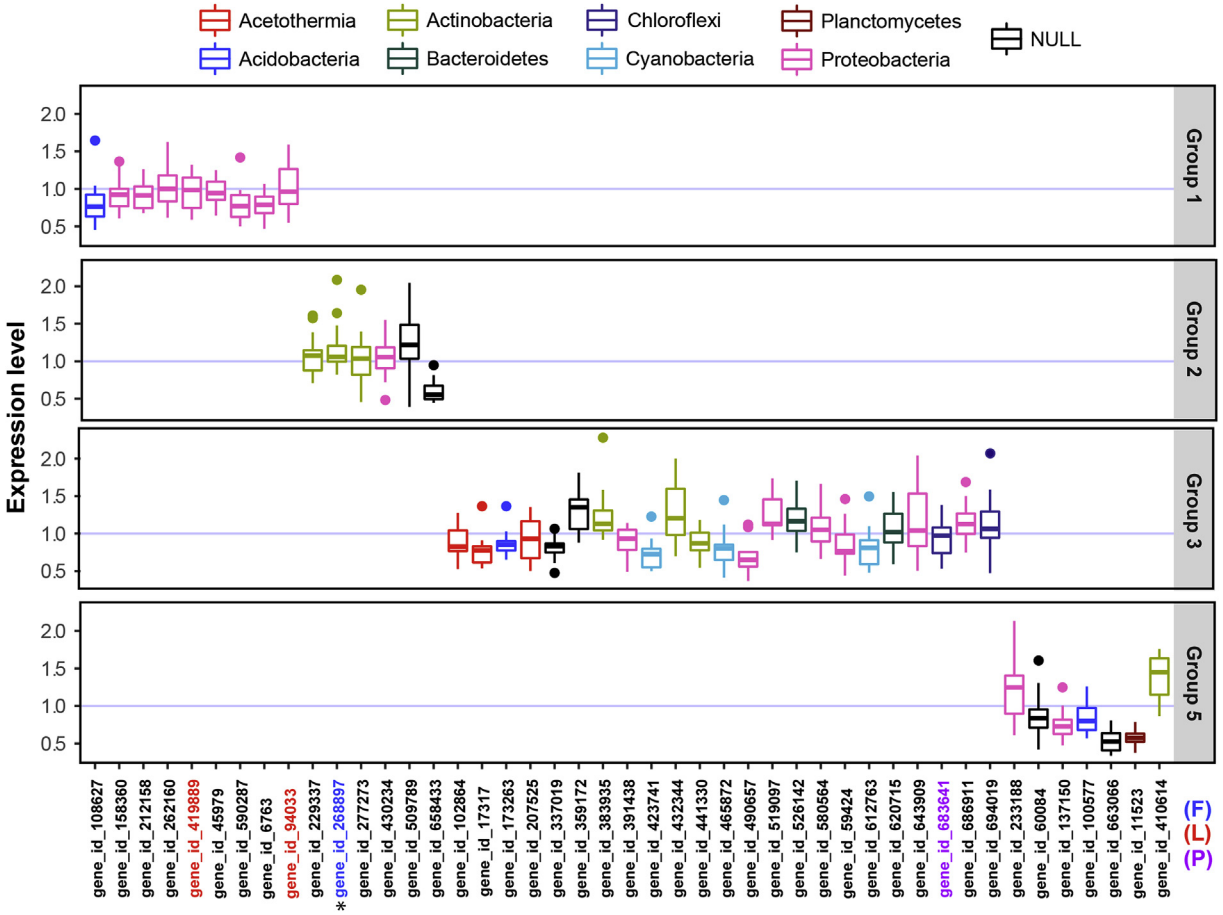

Fig. 1. [NiFe]-hydrogenase gene expression ratio as a function of functional groups. The middle line in the boxes represents the median and the dots reflect outliers. Lower and upper lines delimit the 25% and 75% quantiles, respectively. Each boxplot is colored according to its affiliated phylum. Gene identifier labels are colored according to the land-use type where H_2 exposure led to a significant differential expression ratio (F; Farmland, L; Larch, P; Poplar) and the asterix (*) denotes a significant differential expression in all three-land use types. The shaded gray line marks the separation between over-expressed (greater than 1) and under-expression (lower than 1) levels.

network suggesting a number of classes within each group (Sondergaard et al., 2016).

Here, we test the hypothesis that retrieved hydrogenase gene profiles from *meta*-omics analyses, although incomplete, represent a valuable surrogate of high- and low-affinity HOB activity in soil. We used data from previous experiments in which soil samples collected from a larch plantation, a poplar plantation and a farmland were exposed to low (0.5 ppmv) or elevated (10,000 ppmv) H_2 concentrations (Khdhiri et al., 2017). Low- and high-affinity H_2 oxidation rates, soil physico-chemical properties and metagenomic profiles measured in the previous report were supplemented here with a metatranscriptomic analysis (Method S1). Quality control-passed metatranscriptomic reads were mapped, using the Burrows-Wheeler aligner BWA v0.7.10 (Li and Durbin, 2010), against contigs from the metagenome assembly after alignment sorting with samtools v1.1 (Li et al., 2009) and gene abundance computation with bedtools v.2.17.0 (Quinlan and Hall, 2010). On average, 52% of the metatranscriptomic reads were mapped to the metagenomic contigs (Table S1). The 45 partial genes encoding the large subunit of [NiFe]-hydrogenases retrieved from the previous metagenomic analysis were represented in the metatranscriptomics data set (Table S2A). Comparative analyses were computed using either gene or transcript counts from *meta*-omics analysis or gene expression ratios (Method S1). This case study was well-suited to challenge our hypothesis, as H_2 exposures caused drastic variations in H_2 oxidation rates measured in the three soils, including a 94–96% loss of high-affinity oxidation activity and a 103–514% gain of low-affinity activity in soil upon elevated H_2 exposure (Khdhiri et al., 2017).

Taxonomic analysis of retrieved hydrogenase genes was performed at the phylum level to minimise misclassification errors due to frequent lateral transfer of [NiFe]-hydrogenase (Constant et al., 2011), leading to the identification of potential HOB belonging to 8 distinct phyla, all within the bacterial kingdom (Fig. S1). Unclassified sequences at the phylum level presented a minor proportion, not exceeding 9% of identified genes. Consistent with recent metagenomic database mining (Greening et al., 2016), Acidobacteria represented only a small proportion of HOBs (less than 2%), while Proteobacteria and Actinobacteria were dominant in all land-uses (40% and 25%, respectively). Hydrogenase gene expression ratios were maintained at an even level in Proteobacteria, while an under- and over-expression was observed in Acidobacteria and Actinobacteria, respectively (Fig. S2). The impact of H_2 exposure on hydrogenase gene and transcript did not yield any significant difference (EdgeR, $P > 0.05$). The impact of H_2 on hydrogenase gene expression ratio was tested after clustering [NiFe]-hydrogenase at the phylum-, hydrogenase group- or gene-levels. At the taxonomic level, no phylum showed a significant response to H_2 exposure (Table S2B). At the functional group level, a specific response was expected due to the physiological role of group 2b [NiFe]-hydrogenases in regulating the expression of hydrogenase gene operons according to H_2 availability (Lenz and Friedrich, 1998). Moreover, a significant decrease of group 5 [NiFe]-hydrogenase gene expression was observed in the same soil microcosms as those used in this study using qPCR and qRT-PCR techniques (Piche-Choquette et al., 2017).