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Meta-omics survey of [NiFe]-hydrogenase genes fails to capture drastic variations in H₂-oxidation activity measured in three soils exposed to H₂

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1 Title: Meta-omics survey of [NiFe]-hydrogenase genes fails to capture drastic
2 variations in H₂-oxidation activity measured in soil

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24Abstract

25~~In a debatable framework regarding the use of incomplete portraits of~~
26Microbial community structure and functions obtained from metagenomic
27data are often used to infer biogeochemical processes. ~~H~~ here we used the
28biological sink of H₂ as a case study to test the hypothesis that [NiFe]-
29hydrogenase gene distribution and expression profiles explain variations in H₂
30oxidation rate measured in soils. Shotgun meta-omics (metagenomic and
31metatranscriptomic) analyses of soil samples exposed to elevated or low H₂
32concentration led to the identification of 45 genes encoding the large subunit
33of [NiFe]-hydrogenases belonging to 8 distinct phyla. Our results indicate that
34~~despite significant sequencing effort~~, retrieved hydrogenase sequences are not
35~~relevant in themselves adequate~~ surrogates of H₂ oxidation activity in soil.
36~~Furthermore~~In fact, land-use exerted a ~~more important~~greater contribution
37influence than H₂ exposure on both hydrogenase gene distribution and
38expression, though expression of certain genes responded to H₂. We argue that
39approaches relying on PCR/RT-PCR amplicons sequencing or quantification
40combined with physicochemical parameters are currently the best option to
41infer the activity of H₂-oxidizing bacteria, as well as other specialist functional
42guilds with similar population size in soil.

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44Keywords: Metagenomic, Biogeochemistry, Microbial Ecology, H₂ oxidation
45activities

47Microbial community profiles are increasingly used to refine biogeochemical
48process models relying on abiotic factors (Todd-Brown et al., 2012; Wieder et
49al., 2013; Pérez-Valera et al., 2015; Powell et al., 2015). Nevertheless, the
50appeal of this approach remains controversial. For instance, modeling diverse C
51and N process rates using PCR amplicon sequencing of functional or 16S rRNA
52genes did not improve every single model previously based on edaphic factors
53(Graham et al., 2014; Graham et al., 2016). Thus, in this study we investigate
54the application of meta-omics (metagenomic and metatranscriptomic)
55approaches in molecular biogeochemistry using molecular hydrogen (H₂) as a
56case study. Aerated upland soils are the most important H₂ sinks. Indeed, high-
57affinity H₂-oxidizing bacteria (HOB) are responsible for 70% of global losses of
58tropospheric H₂ (Pieterse et al., 2013) while low-affinity HOB scavenge H₂
59produced by nodules [on plant roots](#), preventing its diffusion to the atmosphere
60(Schuler and Conrad, 1991). According to their physiological role and cellular
61localization, [NiFe]-hydrogenases are classified into 5 distinct functional
62groups. Briefly, group 1 includes membrane-bound hydrogenases supplying
63electrons to the respiratory chain; group 2 are either H₂ sensors regulating the
64transcription of hydrogenases operons or recyclers of H₂ by-products of
65nitrogen fixation; group 3 are used to regenerate reducing equivalents in the
66cells (e.g. NAD, ferredoxin-420); group 4 hydrogenases are membrane-bound
67H₂-evolving enzymes operating under anaerobic conditions (Vignais and
68Billoud, 2007; Greening et al., 2016) and lastly, group 5 encompasses high-
69affinity hydrogenases used for mixotrophic growth and survival (Constant et al.,
702011).

71Here, we test the hypothesis that, ~~albeit incomplete,~~ retrieved hydrogenase
72gene profiles from meta-omics analyses, although incomplete, represent a
73valuable surrogate of high- and low-affinity HOB activity in soil. We used data
74from previous experiments in which soil samples collected from a larch
75plantation, a poplar plantation and a farmland were exposed to low (0.5 ppmv)
76or elevated (10,000 ppmv) H₂ concentrations for 15 days (Khdhiri et al., 2017).
77Low- and high-affinity H₂ oxidation rates, soil physicochemical properties and
78metagenomic profiles measured in the previous report were supplemented with
79a metatranscriptomic analysis. Quality control-passed metatranscriptomic
80reads were mapped, using the Burrows-Wheeler aligner BWA v0.7.10 (Li and
81Durbin, 2010), against contigs from the metagenome assembly after alignment
82sorting with samtools v1.1 (Li et al., 2009) and gene abundance computation
83with bedtools v.2.17.0 (Quinlan and Hall, 2010). In this paper, 45 genes
84encoding the large subunit of [NiFe]-hydrogenases retrieved from the
85metagenomic analysis based on a trained set of Hidden Markov Models (Khdhiri
86et al., 2017) and complementary phylogenetic analysis (Figure S1) were used
87as a template to retrieve corresponding hydrogenase gene transcripts from
88metatranscriptomic profiles (Table S1A). Comparative analyses were computed
89using either gene or transcript counts from meta-omics analysis or gene
90expression ratios (*i.e.* transcript count divided by gene count). This case study
91was well-suited to challenge our hypothesis, as H₂ exposures caused drastic
92variations in H₂ oxidation rates measured in the three soils, including a 94-96%
93loss of high-affinity oxidation activity and a 103-514% gain of low-affinity
94activity in soil upon elevated H₂ exposure (Khdhiri et al., 2017).

95Hydrogenase genes encompassed 8 distinct phyla, all within the bacterial
96kingdom (Figure S2). Unclassified sequences at the phylum level presented a
97minor proportion, not exceeding 9% of identified genes. In accordance with
98recent metagenomic database mining (Greening et al., 2016), Acidobacteria
99represented only a small proportion of HOBs (less than 2%), while
100Proteobacteria and Actinobacteria were dominant in all land-uses (40% and
10125%, respectively). Hydrogenase gene expression ratios were maintained at an
102even level in Proteobacteria, while an under- and over-expression was
103observed in Acidobacteria and Actinobacteria, respectively (Figure S3). The
104impact of H₂ exposure on hydrogenase transcription was analyzed using
105sequencing reads clustered at the phylum, functional group and gene levels. At
106the taxonomic level, no phylum showed a significant response to H₂ exposure
107(Table S1B). At the functional group level, a specific response was expected
108due to the physiological role of group 2B [NiFe]-hydrogenases in regulating the
109expression of hydrogenase gene operons according to H₂ availability (Lenz and
110Friedrich, 1998). Moreover, a significant decrease of group 5 [NiFe]-
111hydrogenase gene expression was observed in those same land-uses using
112qPCR and qRT-PCR techniques (Piche-Choquette et al., 2017). Nevertheless,
113there was no consistent response to H₂ treatment when examining the five
114groups separately (Table S1C). At the gene level, the expression of 3
115hydrogenase genes showed a significant response to H₂ exposure (Figure 1). In
116fact, land-use type exerted more incidence-influence on hydrogenase gene or
117transcript distribution and gene expression than the H₂ treatment (Table S2).

118Bacterial and fungal species richness along with edaphic factors were
119previously reported to contribute significantly in explaining H₂-oxidation rates

120variations in structural equation models (Khdhiri et al., 2017). Here, we added a
121supplementary composite variable representing hydrogenase gene counts
122(Mg), transcript counts (Mt) or gene expression profiles (Mt/Mg) to these
123models to evaluate the contribution of meta-omic datasets in explaining HOB
124activity. The attempt to reduce hydrogenases' molecular profiles into principal
125components failed to reach appreciable explanatory power (Figure S4) and
126therefore, composite variables were computed through the sum of all gene
127counts, transcript counts and gene expression ratio for each sample. Added
128composite variables exerted no significant contribution in explaining low-
129affinity H₂ oxidation activity regardless of the analysis level (Figure 2A, C, E).
130On the other hand, high-affinity activity was partly explained by the composite
131variable Mt (Figure 2D). Under-representation of hydrogenases genes in the
132sequenced metagenomes caused by insufficient sequencing depth is likely a
133critical factor explaining the lack of a significant contribution of meta-omics
134datasets to explain measured activity of HOB. Regarding high-affinity HOB, our
135meta-omics datasets recovered only 7 genes encompassing group 5 [NiFe]-
136hydrogenases (Figure 1), while PCR-amplicon sequencing of *hhyL* gene
137(encoding the large subunit of hydrogenase belonging to group 5) led to the
138identification of 664 OTUs in the same soils (Piche-Choquette et al., 2017).

139Gene distribution and expression captured through meta-omics surveys were
140not representative of the overall activity of HOB populations in soil. H₂
141oxidation activity gradients observed in this study are comparable to extreme
142variations occurring in the environment when comparing different land-uses.
143For instance, potential high-affinity H₂ oxidation rates varied between 100 and
1442000 pmol g_(dw)⁻¹ h⁻¹ in soil samples collected from spruce forest and arid desert,

145respectively (Constant et al., 2011). Similarly, investigation of the impact of
146land-use change on H₂ dry deposition unveiled 32-64% lower H₂ soil uptake
147rate in a grazed pasture when compared to native rainforest in the dry season
148(Pendall et al., 2010). Considering the lack of significant covariation between
149hydrogenase molecular profiles and H₂ oxidation activities under the extreme
150gradient of activities we experienced, we do not expect better resolution of
151HOB activity in natural environments. Thus, we recommend the use of qPCR
152and qRT-PCR techniques in studies aimed at inferring the potential activity of
153HOB in soil. For instance, multiple linear regression parameterized with total
154carbon and the relative abundance of high-affinity HOB explained up to 76%
155variation in high-affinity H₂ oxidation rates measured in 26 soils (Khdhiri et al.,
1562015). Likewise, *hhyL* gene expression ratio analyses explained 45% variation
157of H₂ oxidation activity measured in soil (Piche-Choquette et al., 2017). With
158the increasing size of public (meta)genomic databases and the democratization
159of high-throughput sequencing techniques, observations reported in this study
160are relevant way beyond the scope of the case of the biological sink of H₂.
161Indeed, it is estimated that high-affinity HOB represent approximately 1% of
162soil microbial communities (Khdhiri et al., 2015), which is comparable to the
163abundance of other functional groups in soil including CO- and CH₄-oxidizing
164bacteria (Degelmann et al., 2010; Lalonde and Constant, 2016). In conclusion,
165this work exemplifies that translation of meta-omics profiles into potential
166ecosystem processes or metabolic pathways is hampered by the poor coverage
167of typical sequencing efforts and the uncoordinated response of individual
168members of the same functional guilds to environmental pressures.

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179

180 **Accession numbers**

181 Raw sequence reads of the metatranscriptomic analysis were deposited in the
182 Sequence Read Archive of the National Center for Biotechnology Information
183 under BioProject [PRJXXX](#).

184

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269

Figure Legend

271 **Figure 1.** [NiFe]-hydrogenases gene expression ratio as a function of
272 functional groups. The middle line in the boxes represents the median and the
273 dots reflect outliers. Lower and upper lines delimit the 25% and 75% quantiles,
274 respectively. Each boxplot is colored according to its affiliated phylum. Gene
275 identifier labels are colored according to the land-use type where H₂ exposure
276 led to a significant differential expression ratio (F; Farmland, L; Larch and P;
277 Poplar). The shaded gray line marks the separation between over- and under-
278 expression levels.

279 **Figure 2.** Structural equation models (SEM) to test the hypothesis of a
280 significant contribution of microbial communities' molecular data from the
281 three levels of analysis to the low- and high-affinity H₂ oxidation models.
282 Hexagon variables represent statistical composites estimated based on partial
283 least square linear regression analysis of the respective variables. The R²
284 values are presented for both oxidation rates. The simple path (direct arrow)
285 tests the significance effect between the two variables while double directed
286 arrows denote a covariation between variables. All path coefficients were
287 standardized and were significant if $P < 0.05$. The "ns" represent a non-
288 significant relationship. For each level of analysis both the low- and high-affinity
289 H₂ oxidation activities are respectively shown as A and B for the metagenomics
290 level; C and D for the metatranscriptomics level and E and F for the expression
291 ratio level.

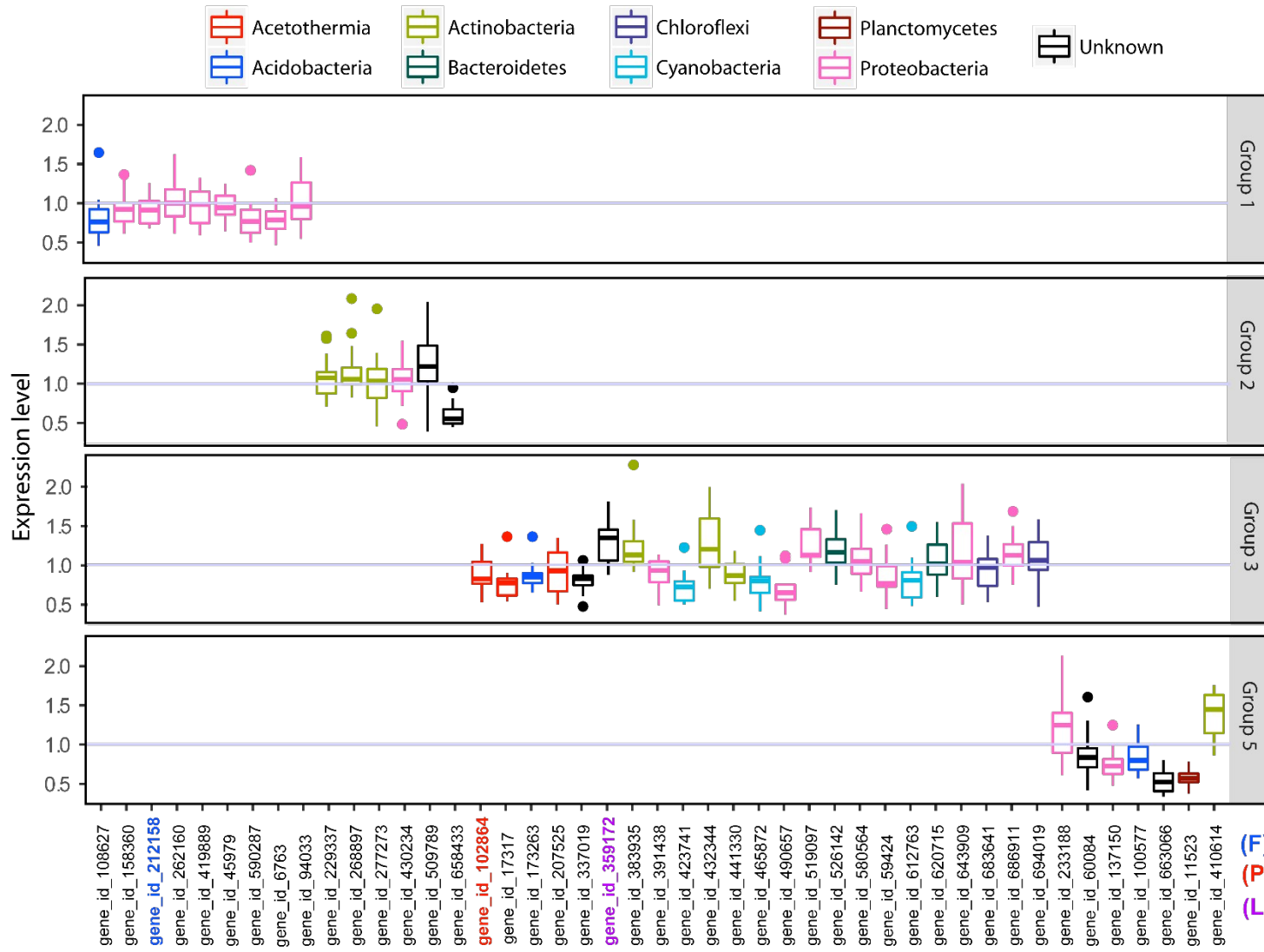
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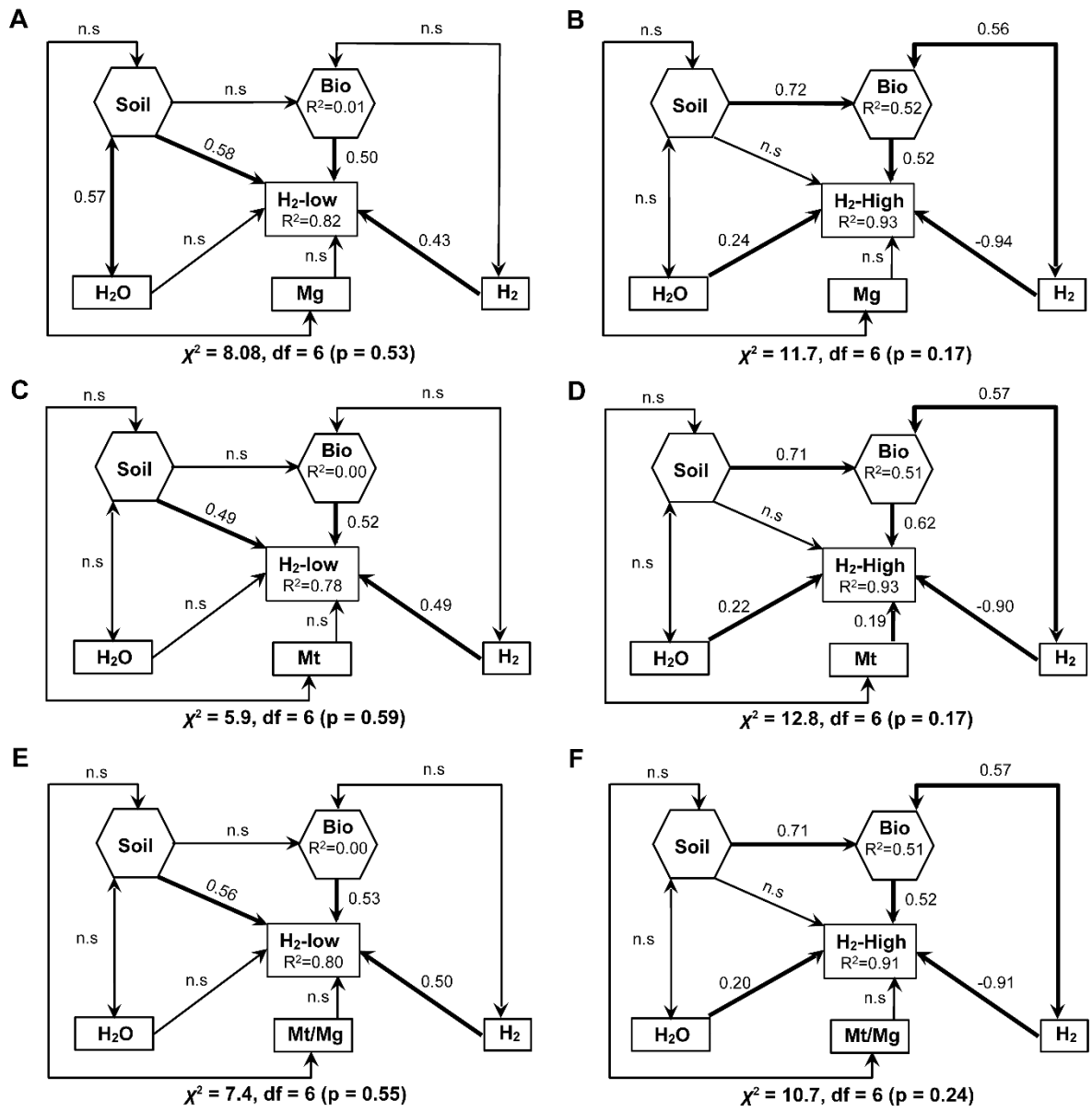
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298 **Figure 1.**



300 **Figure 2.**



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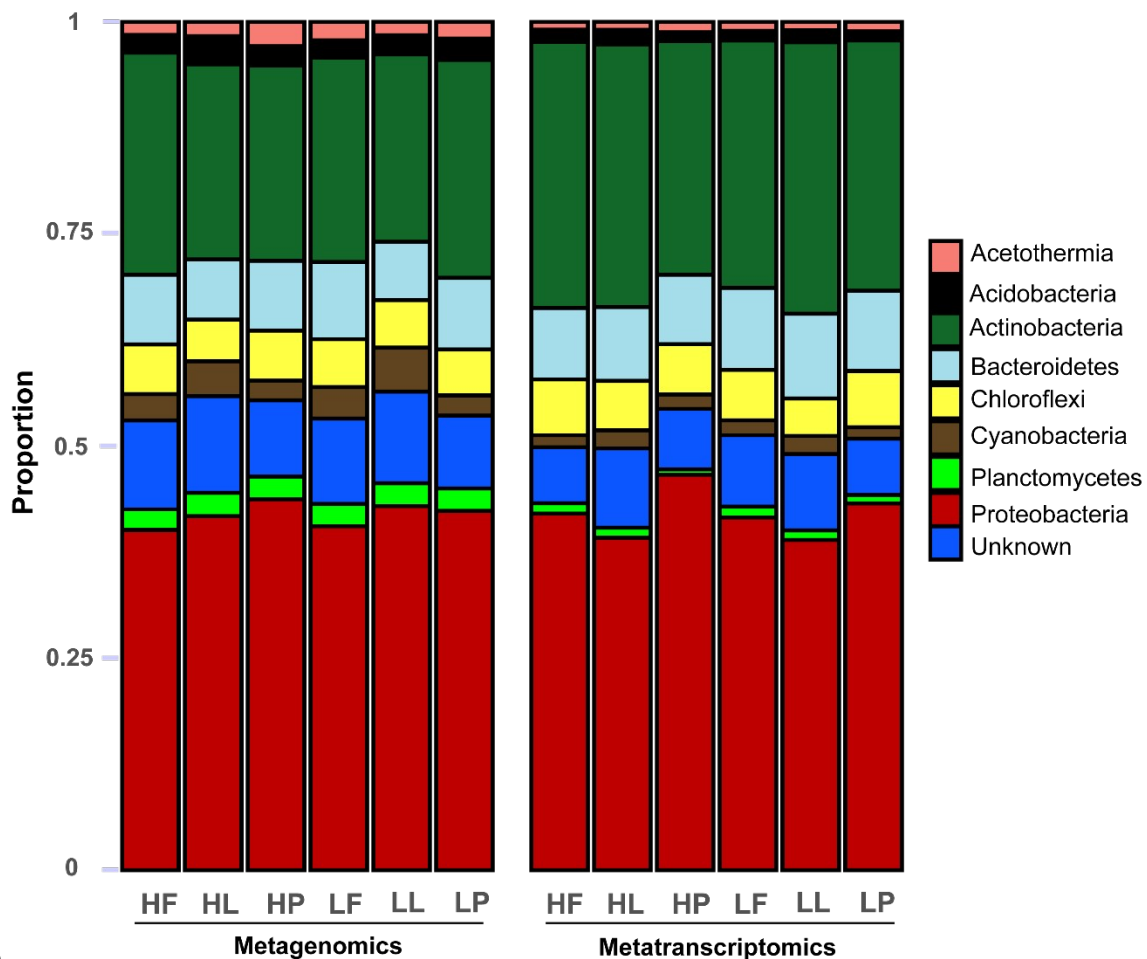
303 **Table S2.** PERMANOVA analysis displaying the proportion of variance in
 304 meta-omic profiles explained by land-use type, H₂ treatment or the
 305 combination land-use type and H₂ treatment.

[NiFe]-hydrogenase dataset	Factors	R² (p-value)
Metagenome	H ₂	0.03 (0.597)
	Land-use	0.37 (0.001)
	H ₂ x Land-use	0.08 (0.460)
Metatranscriptome	H ₂	0.04 (0.353)
	Land-use	0.40 (0.001)
	H ₂ x Land-use	0.07 (0.529)
Expression ratio	H ₂	0.05 (0.675)
	Land-use	0.15 (0.066)
	H ₂ x Land-use	0.11 (0.678)

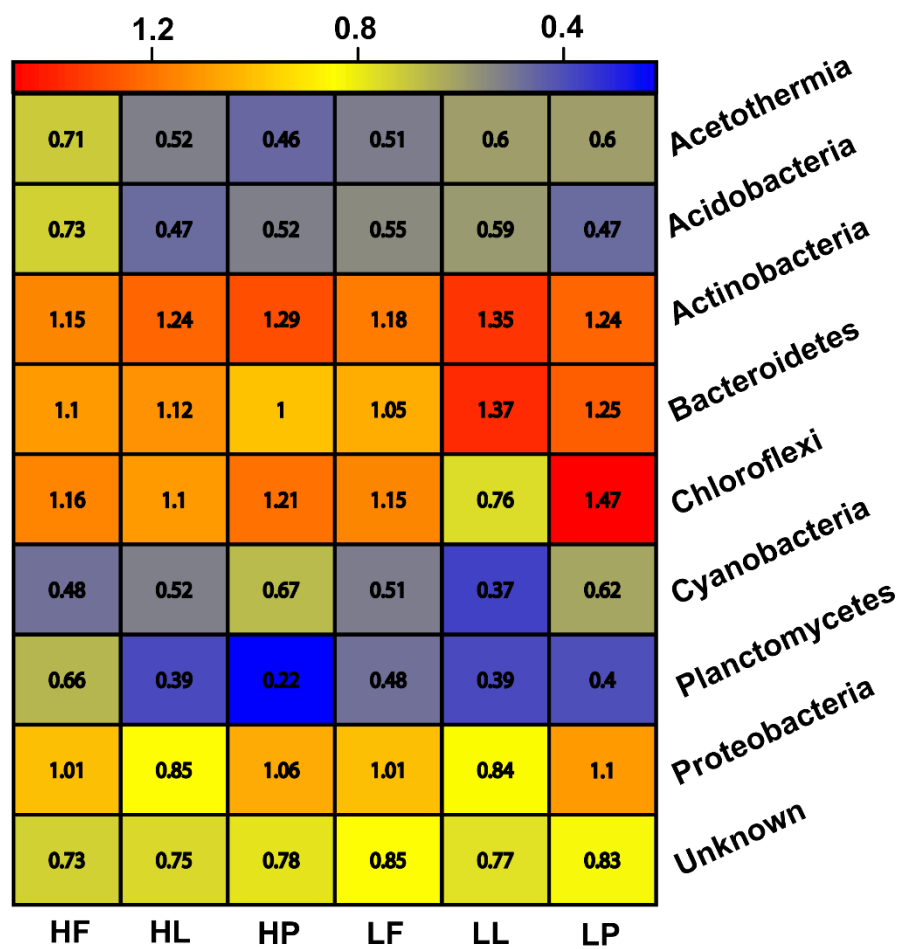
306* Significant R² are shown in bold

307

309 **Figure S2.** Distribution of [NiFe]-hydrogenase genes and transcripts at
 310 the phylum level. The plots represent the sum of relative gene counts for
 311 each phylum. The barplot shows the average value of genes counts from
 312 three replicates of the three soils (F; Farmland, L; Larch and P; poplar)
 313 exposed to elevated (H) or low (L) H₂ concentrations. 18 and 17 samples
 314 were considered for metagenomic and metatranscriptomic libraries,
 315 respectively.



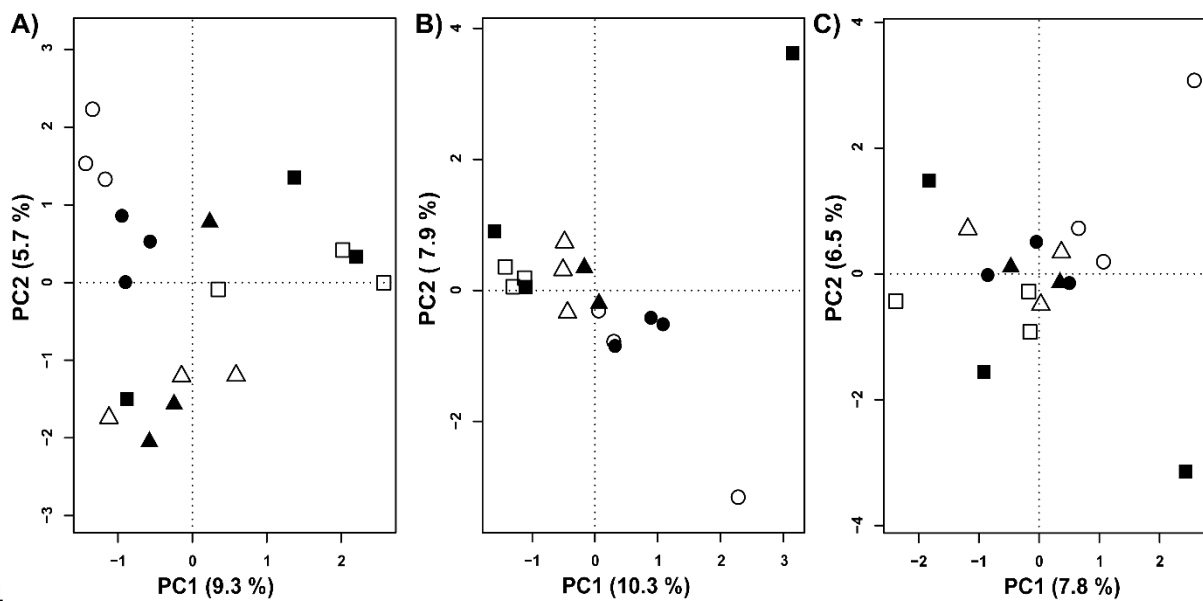
317 **Figure S3.** Heatmap of hydrogenases expression ratios at the phylum
 318 level. Each expression ratio value represents the ratio of the sum
 319 calculated from all genes of their respective phylum in the three replicates
 320 of each treatment condition. The first letter of soil nomenclature denotes
 321 the treatment condition (H and L for high and low H₂ treatment,
 322 respectively) and the second letter refers to the soil land-use (F; Farmland,
 323 L; Larch, P; Poplar).



324

325

326 **Figure S4.** Principal component analysis showing the distribution of
327 different land-use types based on the relative abundance of the 45
328 putative [NiFe]-hydrogenases on A) the metagenomics level, B) the
329 metatranscriptomics level and C) the expression ratios level. Land-use
330 types are represented by three different symbols (squares; Farmland,
331 circles; Larch, triangles; Poplar). Black symbols refer to the high
332 concentration treatment while white symbols indicate soils exposed to low
333 concentration of H₂.



334

335