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Dose-response relationships between environmentally-relevant H_2 concentrations and the biological sinks of H_2, CH_4 and CO in soil

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ABSTRACT

Local H_2 accumulations can be found in soil, especially within legume crop agroecosystems, where H_2 is an obligate by-product of nitrogen fixation. Recent investigations show that diffusive fluxes of H_2 act as additional energy inputs shaping microbial community structure and function in soil. The goal of this study is thus to define dose-response relationships between H_2 exposure and soil microbial community dynamics. Community structure and trace gases (*i.e.* H_2, CH_4 and CO) oxidation activities were investigated following soil incubation to environmentally-relevant H_2 mixing ratios. Despite no evidence of an alteration of microbial diversity, coordinated dose-response relationships between trace gases oxidation rates and H_2 exposure were recorded. Measured H_2 oxidation rates were implemented into a theoretical framework modeling H_2 decay as a function of distance from H_2-emitting point sources. Theoretical H_2 concentration profiles and dose-response relationships between H_2 concentration and trace gases oxidation rates were integrated to predict the impact of H_2 on microbial community functioning. While most H_2 is oxidized within 1 cm of H_2 point sources, trace gases oxidation is predicted to be altered within a 10 cm radius. High-affinity CH_4 and CO oxidation capacities dropped by up to 78% and 84% along H_2 concentration gradients, respectively. Theoretical distances from H_2-emitting point sources required to reactivate 50% of maximal CH_4 oxidation rate were 0.3 cm in farmland soil and 0.2 cm in poplar soil. A longer distance was required to reactivate 50% of CO oxidation rate, *i.e.* 1.5 and 2.7 cm in farmland and poplar soils, respectively. Loss of CH_4 oxidation potential observed under elevated H_2 exposure was correlated with a gain of low-affinity H_2 oxidation activity, while a substrate inhibition of high affinity H_2 oxidation rate was paralleled with a decreasing trend of CO oxidation activity. In addition to shedding light on potential interactions between H_2, CH_4 and CO biogeochemical processes in soil, these novel findings provide evidence that H_2 supports metabolic and energetic flexibility in microorganisms supplying a variety of ecosystem services.

1. Introduction

Biological N_2-fixation (BNF) is performed either in N_2-fixing nodules (Mus et al., 2016) or by free-living N_2-fixing bacteria (Orr et al., 2011) according to the following enzymatic reaction: $N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$ (Hoffman et al., 2009). While providing bioavailable N to surrounding organisms, BNF also produces substantial amounts of H_2 as an obligate by-product. The high density of N_2-fixing bacteria in nodules leads to a local accumulation of H_2, with H_2 mixing ratios ranging from 9000 to 27 000 ppmv in nodules (Hunt et al., 1988; Witty, 1991; Witty and Minchin, 1998), of which diffusion losses can account for up to 240 000 L H_2 per hectare of legumes per growing season (Dong et al., 2003). Metabolic capabilities of rhizobacterial symbionts recruited by plants represent the main determinants of the intensity of these H_2 emissions in soil. Indeed, plant roots can establish symbiosis with rhizobacteria displaying either a *Hup*$^+$ or *Hup*$^-$ genotype, according to the presence or absence of an uptake [NiFe]-hydrogenase, respectively. *Hup*$^+$ rhizobacteria have an energetic advantage since their [NiFe]-hydrogenase enables them to recycle the energy potential of H_2 that would otherwise be lost during N_2 fixation. In *Hup*$^-$ legumes rhizosphere, H_2 is thus released into surrounding soil, resulting in a net loss of energy at the plant-nodule level, which roughly translates to 5–6% of net photosynthesis energy output (Dong and Layzell, 2001). At first glance, the existence of this genotype looks like a flawed symbiosis, yet analyses of N_2-fixing nodules *in situ* have shown that less energy-efficient nodules, *i.e.* *Hup*$^-$ nodules, were more widespread than their *Hup*$^+$ counterpart (Uratsu et al., 1982; Dong et al., 2003). This is especially true in annual crops without well-established root systems (Annan et al., 2012). A potential argument supporting preferential recruitment of less energy-efficient plant symbionts is the fact that H_2 released into surrounding soils, analogously to *Hup*$^-$ nodules, has been shown to increase plant

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biomass by 15–48% in both legumes and non-legumes (Dong et al., 2003). Although the exact mechanisms behind H_2 fertilization remain elusive, enrichment of Knallgas bacteria, *i.e.* aerobic H_2-oxidizing bacteria (HOB), including plant growth-promoting rhizobacteria (PGPR) showing either 1-aminocyclopropane-1-carboxylate (ACC) deaminase or rhizobitoxine activity promoting nodulation and root elongation, was proposed (Maimaiti et al., 2007). *In situ*, H_2 is quickly used up by HOB, within a 3–4.5 cm radius from the soil-nodule interface (Lafavre and Focht, 1983). The magnitude of this biological sink is supported by the negligible amount of H_2 escaping from legume fields to the atmosphere (Conrad and Seiler, 1979). While N_2-fixing nodules and their underlying plant-microbe interactions have been extensively studied over the years (Dixon and Kahn, 2004; Mus et al., 2016), the spatial patterns of microbial communities and processes along their resulting H_2 gradients has received little attention.

As a potent energy source for soil microbial communities, diffusive H_2 fluxes released by *Hup*$^-$ nodules are indubitably expected to stimulate H_2-oxidizing microbes such as Knallgas bacteria, with K_m *ca.* 1000 ppmv, and high-affinity HOB, with K_m *ca.* 100 ppmv (Schuler and Conrad, 1990; Häring and Conrad, 1994; Dong and Layzell, 2001; Piché-Choquette et al., 2016). Knallgas bacteria can quickly oxidize high loads of H_2, enabling them to use it as an energy source for facultative chemolithoautotrophic growth. However, the low-affinity of their [NiFe]-hydrogenase enzymes prevents them from using H_2 below a specific threshold concentration, namely at atmospheric mixing ratios (Conrad et al., 1983). Therein, high-affinity HOB, possessing unusual high-affinity [NiFe]-hydrogenases, take over the H_2-oxidizing process for their own maintenance energy (Constant et al., 2010; Greening et al., 2014; Liot and Constant, 2016) at low H_2 concentrations. The stimulation of these contrasting HOB sub-populations along H_2 concentration gradients is expected to result in a differential alteration of surrounding soil microbial communities, notably considering that HOB are broadly distributed in both bacterial and archeal domains of life (Vignais and Billoud, 2007; Greening et al., 2016; Piché-Choquette et al., 2017) and thus, can be responsible for a wide variety of ecological functions apparently unrelated to H_2-oxidation.

Previous investigations have shown that elevated H_2 mixing ratios reflecting those found in natural ecosystems could indeed result in a shift in soil microbial communities' structure (Stein et al., 2005; Zhang et al., 2009; Osborne et al., 2010; Piché-Choquette et al., 2016; Khdhiri et al., 2017) and more importantly, in a coordinated feedback of communities' functions (Khdhiri et al., 2017). Soil exposure to 10 000 ppmv H_2 led to, amongst other responses, a 2- to 4-fold decrease in CH_4 oxidation potential and a diversification in carbon utilization potential (Khdhiri et al., 2017). Considering steep H_2 concentration gradients found in the environment, the present study aims to define dose-response relationships between H_2 exposure and the alteration of soil microbial community structure and function. For this purpose, soil microbial community structure and trace gases oxidation rates were investigated in soil exposed to various H_2 mixing ratios encompassing gradients found in natural environments. These observations were implemented into a theoretical framework modeling the decay of H_2 mixing ratios as a function of distance from H_2-emitting point sources, therein built according to Fick's second law of diffusion while also taking microbiological constraints into account. Dose-response relationships between H_2 mixing ratios and microbial processes were finally used to model H_2, carbon monoxide (CO) and methane (CH_4) oxidation rates as a function of distance from H_2-emitting point sources. Experimental observations and theoretical frameworks provide new experimental evidence into the potential impact of H_2 on soil biogeochemical processes.

2. Material and methods

2.1. Soil sampling and microcosms incubation

Soil samples were collected from a farmland and a poplar plantation. Site location, sampling protocol, soil physicochemical properties and the dynamic microcosm chambers unit are all described in (Khdhiri et al., 2017). Briefly, soils were air-dried and sieved (2 mm mesh size) prior to their incubation in a dynamic microcosms chamber unit comprising a programmable gas mixer enabling exposure of up to seven independent soil microcosms to a user-defined H_2 mixing ratio. Incubations were performed under a systematic experimental design in which six experimental units, consisting of three independent replicates of farmland and poplar soil microcosms, were exposed to one of the five selected H_2 exposure treatments (0.5, 50, 500, 5 000, and 10 000 ppmv H_2). Both H_2 exposure treatment and position of each microcosm inside the dynamic microcosm chamber unit were randomly selected between each set of incubation. Each incubation lasted for 15 days before the destructive sampling of microcosms for microbial communities and trace gases oxidation activity measurements. Soil subsamples dedicated to microbial community structure analyses were stored at −80 °C until DNA extraction was performed, while other analyses were processed immediately.

2.2. Nucleic acids extraction and purification

Soil DNA and RNA were co-extracted using a protocol based on (Mettel et al., 2010), with a few exceptions to the original protocol detailed as follows. 35 µL 20% SDS (sodium dodecyl sulfate) was added to each 0.5 g soil sample alongside the TPM buffer. Microbial cells were lysed by bead-beating using a FastPrep®-24 homogenizer (MP Biomedicals, Solon, USA) at 6.5 m s^{-1} for 45 s. All centrifugation steps before nucleic acids precipitation lasted for 5 min at 4 °C rather than 1 min. The pH 4.5 phenol-isoamyl alcohol used in the first extraction step was supplemented with 1 g L^{-1} 8-hydroxyquinoline as anti-oxidant. For the precipitation step, 0.1 vol 3.0 M sodium acetate (pH 5.2) was first added to the supernatant, followed by 2.0 vol 95% ethanol. Samples were then slightly shaken and were left to precipitate for 12 h at −20 °C. Samples were then centrifuged at 20 000 g for 60 min at 4 °C, pellets were washed twice with 70% ethanol and were resuspended as stated in the original protocol. RNA was preserved for other studies, while DNA was treated with 5 µL 100 µg ml^{-1} RNaseA and left at room temperature for 15 min. DNA was then purified using acid-washed polyvinylpolypyrrolidone columns according to (Holben et al., 1988). DNA samples were assessed for integrity on a 1.0% agarose gel and quantified using Quantifluor dsDNA System® (Promega, Fitchburg, WI, U.S.A.) and a Rotorgene 6000® thermocycler (Qiagen, NRW, Germany). Nucleic acids were preserved at −80 °C until sent for sequencing.

2.3. PCR amplicon sequencing and sequences analysis pipeline

PCR amplification of the V6-V8 regions of bacterial 16S rRNA gene was performed with B969F-CS1 (5′-ACGCGHNRAACCTTACC-3′) and BA1406R-CS2 (5′-ACGGGCRGTGWGTRCAA-3′) (Comeau et al., 2011) primers, while the ITS2 region of fungal rRNA was PCR-amplified with ITS3_KYO2 (5′-GATGAAGAACGYAGYRAA-3′) and ITS4_KYO3 (5′-CTBTTVCCKCTTCACTCG-3′) (Toju et al., 2012) primers. Both sequencing library preparation and Illumina Miseq reactions (2 × 250 paired-ends configuration) were performed at McGill University Génome Québec Innovation Centre (Montreal, Quebec, Canada). Raw sequencing reads were processed using Usearch10 (Edgar, 2010). In short, paired-ends were first merged to a total length of 400–500 bases, for bacteria, or 200–450 bases, for fungi, to account for the variability in sequence length among sequenced regions. Maximum overlap mismatches accepted for downstream processing consisted of 5 bases or 10%, whichever came first. Primers were trimmed from merged reads, while