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Optimizing measurement of soil potential high-affinity H_2 uptake activity across pH

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Abstract

Soil high-affinity H_2 uptake activity can be affected by many factors, including the soil pH. However, the method to determine how pH affects high-affinity H_2 uptake activity should be updated. The effect of pH on the biological high-affinity H_2 uptake in agricultural soils was compared using three pH buffer systems in the pH 4–8 range. Soil pH was adjusted to the target pH using a buffer system (1 g soil/5 mL pH buffer). Soil slurries were treated with heat (autoclaving) or a chemical (25% v/w of toluene addition, microbial inhibitor) to inhibit biological activity. Sterile pH buffer was used as a negative control. The sterile soil slurry (heat sterilization) was the optimal reference control for measuring biological high-affinity H_2 uptake activity. Biological H_2 uptake activity was resistant to toluene, particularly at extreme pH levels. Overall, soil pH ($p = 0.95$) and pH buffer systems ($p = 0.46$) did not affect the high-affinity H_2 uptake activity in the tested agricultural soils. We provide an updated method to accurately measure the potential high-affinity H_2 uptake activity in soil, with an emphasis on the importance of controlling the soil pH.

Plain Language Summary

Molecular hydrogen (H_2) is the second most abundant oxidizable trace gas in the atmosphere after methane. It is removed from the atmosphere and converted to water by a group of natural soil microorganisms called H_2 oxidizers. The high-affinity H_2 oxidizers, which can metabolize low concentrations of H_2, are a diverse microbial group. Soil bacteria capable of high-affinity H_2 oxidation are widespread in agricultural soils. Furthermore, they function optimally in soil slurries with pH 4–8, regardless of the buffer system in the slurry. This suggests that H_2 oxidation is a ubiquitous and prevalent biological process in soil, regardless of the pH in agricultural fields.

1 | INTRODUCTION

Molecular hydrogen (H_2) is the second most abundant oxidizable trace gas in the atmosphere after methane (CH_4). The tropospheric concentration of H_2 has nearly doubled in the past 150 years and is primarily produced by the oxidation of the atmospheric hydroxyl radical ($OH^\bullet$) with methane (Constant et al., 2009). H_2 is an indirect greenhouse gas because it increases the atmospheric lifetime of methane, a potent greenhouse gas. The environmental concentration of H_2 is controlled by abiotic and biotic sinks that convert $H_2 \rightarrow 2H^+ + 2e^-$. The abiotic sink for H_2 is atmospheric $OH^\bullet$, which accounts for the removal of ~20% of H_2 from the atmosphere annually (Constant et al., 2009). The terrestrial biotic sink for H_2 is microbial oxidation (by soil prokaryotes), which can remove ~80% of the atmospheric H_2 each year (Ehhalt & Rohrer, 2009; Greening et al., 2015; Liot & Constant, 2016). Soil H_2 oxidation varies across land uses; for example, it was greater in deciduous forest > grassland > agricultural > desert soils (Jordaan et al., 2020; Khdhiri et al., 2015). However, H_2 oxidation in soil is primarily a biological process because the soil H_2 uptake was inhibited by the addition of chloroform, a microbial inhibitor. The "free H_2 uptake enzymes" concept was proposed to explain how ~80% of H_2 uptake was lost in chloroform-fumigated soil (King, 2003). Later research demonstrated that <2% H_2 oxidation was possible in bacteria-free soil extracts, compared to bacteria-colonized soil extracts (Guo & Conrad, 2008), refuting the existence of "free H_2 uptake enzymes." Instead, it was proposed that the soil uptake of trace H_2 was primarily a biological process facilitated by high-affinity H_2-oxidizing bacteria that use trace amounts of H_2 as an energy source (Constant et al., 2011; Greening et al., 2016). The existence of $OH^\bullet$ and other abiotic exogenous electron acceptors in soil can also contribute to soil H_2 oxidation. This means that high-affinity H_2 oxidation activity in soil is the sum of the total H_2 oxidation that comes from abiotic and biotic oxidation reactions. Therefore, the potential high-affinity H_2 uptake in soil may be overestimated by measuring total H_2 oxidation, which is the standard method at present.

The biological process of soil H_2 uptake is microbially mediated. Microorganisms responsible for biological H_2 oxidation in soil have variable activity, depending on factors such as soil pH, moisture, temperature, H_2 availability, and soil carbon content (Bertagni et al., 2021; Constant et al., 2009; Greening et al., 2014). Soil pH is an important because it strongly influences microbial functions by influencing nutrient solubility, substrate availability, and enzymatic activity. However, the method of assessing the soil pH effect on H_2 oxidation was not validated for agricultural soil, although agriculture is a significant land use at a global scale. For example, acidic forest soil (pH 5) reached the maximal high-affinity H_2 oxidation rate (0.4 nL H_2 consumed g^{-1} dry soil min^{-1}) at pH 5, with optimal H_2 oxidation at pH 4–6 in forest soil (Guo & Conrad, 2008), whereas the maximum high-affinity H_2 oxidation rate (0.23 nL H_2 consumed g^{-1} dry soil min^{-1}) was observed at pH 8 in neutral garden soil (pH 7.4) (Schuler & Conrad, 1991). These studies indicate that the high-affinity H_2 oxidation rate was about 1.7 times greater in acidic forest soil than in garden soil and suggest that distinct biological processes are related to pH or other soil physicochemical properties. More importantly, Schuler and Conrad (1991) used a spray of strong acid and alkaline solutions to directly rewet the air-dried soil to a specific moisture content and simultaneously adjust the soil pH. The use of strong acids or bases may adversely affect soil microorganisms, and the volume of HCl-NaOH solution used to adjust soil moisture may be insufficient to achieve the intended pH due to the soil's inherent buffering capacity. A robust method of manipulating agricultural soil pH is needed to understand the relationship between soil pH and H_2 uptake activity and to study the ecology of H_2-oxidizing bacteria in agricultural soil.

Core Ideas

- Sterile soil slurry shows no biological H_2 uptake activity across pH gradients.
- H_2-oxidizing microbial community resisted toluene inhibition in acidic and alkaline slurries.
- Soil pH buffer systems do not affect soil high-affinity H_2 uptake activity.

The objective of the present study was to optimize a method to evaluate the effect of soil pH on biological high-affinity H_2 uptake activity by (1) selecting a suitable reference control for the biotic H_2 oxidation in soil and (2) choosing a reliable pH buffer system to reduce the variation caused by buffer salts. It is hypothesized that (1) the sterile treatment will be the best control because sterilization eliminates biological activity without altering the soil abiotic properties, and (2) the buffer system influences high-affinity H_2 uptake activity because buffer salts can be metabolized and thereby affect the microbial activity.

2 | MATERIALS AND METHODS

2.1 | Soil sampling sites

Agricultural soil (5- to 15-cm depth, ~300 g) was collected by random sampling of four fields at the Macdonald Campus Farm of McGill University in Ste-Anne-de-Bellevue, QC, Canada (45°25′ N, 73°55′ W) in November 2022. Agricultural

TABLE 1 Characteristics of the agricultural soils studied for potential high-affinity H_2 uptake activity.

Field number	Soil texture	Soil pH	Total organic carbon (g kg^{-1}, $n = 5$)	Total nitrogen (g kg^{-1}, $n = 5$)
#105	Humic Gleysol, fine sandy loam	6.7	15 ± 0.7	1.2 ± 0.4
#108	Humic Gleysol, clay loam	6.6	10 ± 0.7	0.8 ± 0.1
#124	Humic Gleysol, loam	6.4	21 ± 0.7	2 ± 0.1
#205	Humic Gleysol, clay	6.1	17 ± 0.7	1.6 ± 0.1

Note: Data are the mean ± standard error.

field #105 was planted with soybean, #108 with perennial alfalfa and grass, #205 with perennial switchgrass, and #124 with perennial alfalfa. Soil was transported on ice and refrigerated as field-moist samples (from 11% to 19% gravimetric moisture content) until use. All soil samples were sieved through a 2 mm mesh and incubated at room temperature (22˚C) in the dark for 1 month before analysis. Original soil moisture was maintained by spraying the soil with tap water every week.

Soil pH was determined in air-dried soil: water (1:2, w/v) suspensions using an Accumet AR10 pH meter (Fisher Scientific). The total organic C and N contents were determined in dried (55˚C for 48 h) and ground (<1 mm) soil using a Thermo Finnigan Flash EA 1112 Series C/N Analyzer (Carbo Erba). Characteristics of the studied soil samples are given in Table 1.

2.2 | Preparation of the buffer systems

Three pH buffer systems were prepared. Buffer 1 was a 0.1 M citric acid monohydrate and 0.2 M Na_2HPO_4 buffer system having a wide pH buffer range (from pH 4 to 8). The pH was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, or 8.0 (accuracy ± 0.01 pH unit). Buffer 2 was a 0.1 M citric acid monohydrate and 0.1 M trisodium citrate buffer system that covered a low pH range (from pH 4 to 6); the target pH values adjusted to 4.0, 4.5, 5.0, 5.5, or 6.0. Buffer 3 was a 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4 buffer system that also covered a high pH range (from pH 6 to 8). The target pH was adjusted to the desired pH values 6.0, 6.5, 7.0, 7.5, or 8.0. All chemicals were obtained from Fisher Scientific unless otherwise stated. The target pH of all buffer systems was adjusted using 2 M HCl or 10 M NaOH.

2.3 | Optimizing soil pH adjustment and determining the reference control

Soil sampled from field #105 was used to determine the optimal pH adjustment and to test the reference control. The nonsterile soil was adjusted from pH 4.0 to 8.0 (in pH 0.5 increments) using Buffer 1 (wide pH range) by adding 1 g of moist soil to 5 mL of buffer in a 125 mL Wheaton bottle. The pH of the soil slurry remained at ± 0.05 of the target value during analysis. As soon as the pH buffer was added, the bottle containing ambient air was sealed. Then H_2 was injected into the headspace following Baril and Constant (2023), and the soil buffer was mixed for 20 min on a rotary shaker (150 rpm) at 22˚C in the dark. To distinguish the biological H_2 oxidation from abiotic H_2 oxidation three abiotic controls with inhibited biological activity were used to establish the baseline high-affinity H_2 uptake activity. Control (1) was a soil slurry containing 25% (v/w) toluene (Fisher Scientific), which served as a microbial inhibitor. Toluene is a highly toxic organic solvent and it is widely used to inhibit microbial activity (Inoue & Horikoshi, 1989). Control 2 was a sterilized soil slurry that was autoclaved at 121˚C for 60 min, stored in a biosafety cabinet for 24 h at room temperature (22˚C), and then autoclaved again at 121˚C for 60 min to eliminate newly germinated spores. Control 3 contained 6 mL of sterilized buffer (autoclaved at 121˚C for 60 min) for each pH treatment. High-affinity H_2 uptake rate was determined in nonsterile soil and in the three controls, which involved 144 samples consisting of nine pH gradients (three controls + one non-sterilized soil) and four technical replicates. The biological H_2 uptake was calculated as the measured H_2 uptake rate in nonsterile soil minus that in the abiotic control.

2.4 | The effect of toluene on microbial activity across a wide pH range

The inhibitory effect of toluene on microbial activity was assessed in relation to soil CO_2 respiration, with soil from field #105 only in this experiment. This soil was adjusted to pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 using Buffer 1. Specifically, 7.5 g of 2-mm mesh-sieved air-dried soil was rewetted with 2.5 mL of Buffer 1. The final adjusted pH was 4.0, 4.5, 5.0, 5.3, 5.6, 6.5, 7.0, 7.5, and 8.0. The effect of toluene was tested by adding 25% (v/w) toluene to the pH-adjusted soil (Sullivan & Havlin, 1992; Tabatabai & Bremner, 1969). The toluene-treated soil and the soil without toluene treatment were incubated in 125 mL Wheaton bottles sealed with an aluminum cap at 25˚C in the dark. After 24 h of

incubation, 20 mL of headspace gas was collected from each sample using a gastight syringe and was stored in 12 mL exetainers before analyzing the CO_2 concentration using a Bruker 450 gas chromatograph (Bruker Corporation). The total sample size of the toluene inhibition experiment was 54 samples comprising nine pH treatments × two (toluene + no toluene) × three technical replicates.

2.5 | Soil testing with pH buffer systems

Four agricultural soils (from fields #105, #108, #124, and #205, Table 1) were treated separately with the three prepared pH buffers before determining their potential high-affinity H_2 uptake activity. The purpose of this experiment was to assess the effect of pH buffer salts on the high-affinity H_2 uptake activity. The selected pH range represented the actual soil pH of agricultural fields found in eastern Canada (Canadian Soil Information Service, 2021).

2.6 | Potential high-affinity H_2 uptake activity in soil

High-affinity H_2 uptake rate was measured at 22°C using gas chromatography (Baril & Constant, 2023). The five H_2 concentrations in the headspace of experimental bottles were monitored for 20 min. The slope of the linear models is reported as the measured H_2 uptake rate (ppbv min^{-1}). Briefly, experimental bottles with soil were sealed with septa-installed screw caps before analysis. The initial H_2 concentration in the headspace was adjusted to ~3 ppmv (mL m^{-3}) by injecting ~1 mL of standard H_2 gas mixture (525 ± 10 ppmv H_2, GST-Welco) into the headspace. This initial H_2 concentration in the headspace targeted high-affinity H_2 oxidation (Km < 100 ppm) that can oxidize low H_2 concentrations, especially atmospheric level (Constant et al., 2008; Piché-Choquette et al., 2016). The high-affinity H_2 uptake activity in soil (nmol H_2 consumed g^{-1} dry soil h^{-1}) was calculated from the slope of the measured H_2 uptake rate (ppbv min^{-1}) versus time adjusted with the ideal gas law and soil dry mass (Supporting Information S1 and S2). The microbial-mediated fraction of potential high-affinity H_2 uptake activity was calculated as the difference in H_2 uptake activity measured in the nonsterile soil slurry and the control soil slurry (Table S1) for each pH treatment.

2.7 | Statistical analyses

The difference between pH treatments was determined by one-way analysis of variance using "Rmisc" R package (Version 1.5.1). Linear relationships between the pH treatment and the high-affinity H_2 oxidation activity, the quadratic relationship of CO_2 respiration, and the effect of toluene on high-affinity H_2 oxidation activity across the entire pH gradient were fitted using the "lm" function of the "stats" package. Figures were produced with the "ggplot2" package (Version 3.4.4). The correlation between variables was calculated with the "ggpubr" package (Version 0.6.0). Statistical analyses and plots were generated using R software version 4.3.3 (R Core Team, 2024). Data are reported as the mean ± standard error (SE) unless otherwise stated. The statistically significant level was set at $p < 0.05$.

3 | RESULTS

3.1 | Determination of the control for the high-affinity H_2 uptake measurement

Based on H_2 consumption, the high-affinity H_2 uptake activity in nonsterile field soil and three abiotic controls was assessed across a pH gradient produced in slurries with pH-adjusted buffer systems. As expected, the nonsterile field soil had the greatest high-affinity H_2 uptake rate, compared to controls, and was represented by an inverted U-shaped pH-response curve (Figure 1). The high-affinity H_2 uptake rates were significantly lower in three controls ($p < 0.001$; Table S2). However, soils with 25% toluene addition significantly inhibited the high-affinity H_2 uptake rates ($p < 0.001$) from pH 5.0 to 6.0 only, with no toluene inhibition in the acidic and alkaline pH treatments. The sterile buffer (Control 3) and sterile soil slurry (Control 2) responded similarly, with no difference across the entire pH range ($p = 0.267$; Figure S1; Table S3), but the average H_2 uptake rate in sterile soil slurry (Control 2) was slightly higher than in sterile buffer only (Control 3).

3.1.1 | The effect of toluene on microbial activity in soil with a wide pH range

The effect of toluene on soil microbial activity across a wide pH range was investigated by measuring the CO_2 respiration with and without the addition of 25% toluene. Toluene addition significantly inhibited soil CO_2 respiration compared to nonsterile soil at each tested pH level ($p < 0.001$) (Figure 2). However, toluene could not fully inhibit microbial activity at each pH level. The highest inhibition rate of 91% was observed around pH 6. In acidic and alkaline slurries, the inhibition rates decreased to approximately 70%. These results indicate that soil microbial activity is not completely inhibited by 25% toluene addition and that parts of the microbial community may be resistant to toluene in acidic and alkaline soils.

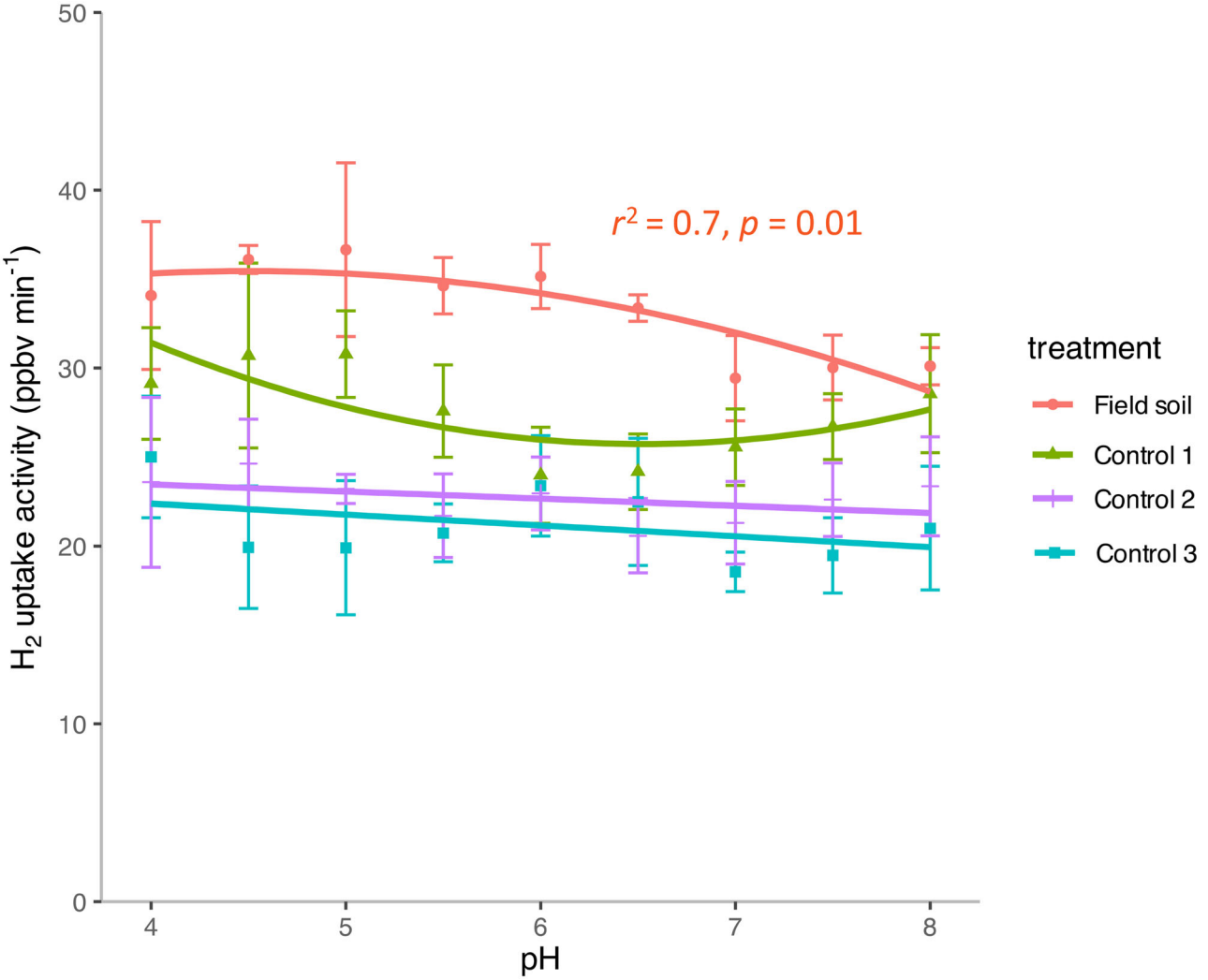

FIGURE 1 The measured soil H_2 uptake rate (ppbv min^{-1}) across pH gradient in agricultural soil (field #105 only) and three controls. The red symbols represent the nonsterile field soil. Control 1 (green symbols) represents the H_2 uptake rates of soils with 25% toluene addition. Control 2 (purple symbols) represents the sterile soil slurry control, and Control 3 (blue symbols) represents sterile buffer control. The data are presented as mean ± standard error ($n = 4$). The significant regression curve was determined using the best-fit quadratic model. Non-significant curves are not shown.

3.1.2 | The effect of toluene on high-affinity H_2 uptake activity in soil with wide pH range

Amending soil with 25% toluene inhibits high-affinity H_2 uptake activity (Figure 3). The pH-response showed that the high-affinity H_2 uptake activity of soil with pH 4.5–7.5 was significantly inhibited (concave upward) by toluene addition ($p < 0.001$; Table S4). The soil with an adjusted pH 6 had high-affinity H_2 uptake activity inhibited by 87%. The acidic pH 4 and alkaline pH 8 had the lowest inhibition of 38% and 23%, respectively (Figure 3a). The variation of high-affinity H_2 uptake activity in toluene-amended soil suggests that the H_2-oxidizing microbial community is resistant to this microbial inhibitor (Figure 3b). Therefore, the composition of the H_2-oxidizing microbial community in acidic and alkaline soils may be different than that in neutral pH soil.

3.2 | The effect of pH buffer system on biological H_2 oxidation

The effects of Buffer 1 (wide pH range), Buffer 2 (acidic pH range), and Buffer 3 (alkaline pH range) buffer systems on high-affinity H_2 oxidation are summarized in Figure 4. No significant regression models could be fitted to the data of any buffer system, across their corresponding pH range.

In general, the H_2 oxidation activity was relatively higher at pH 7 than at other tested pH values, although the best-fit model was not significant with a high-range buffer. Two-way

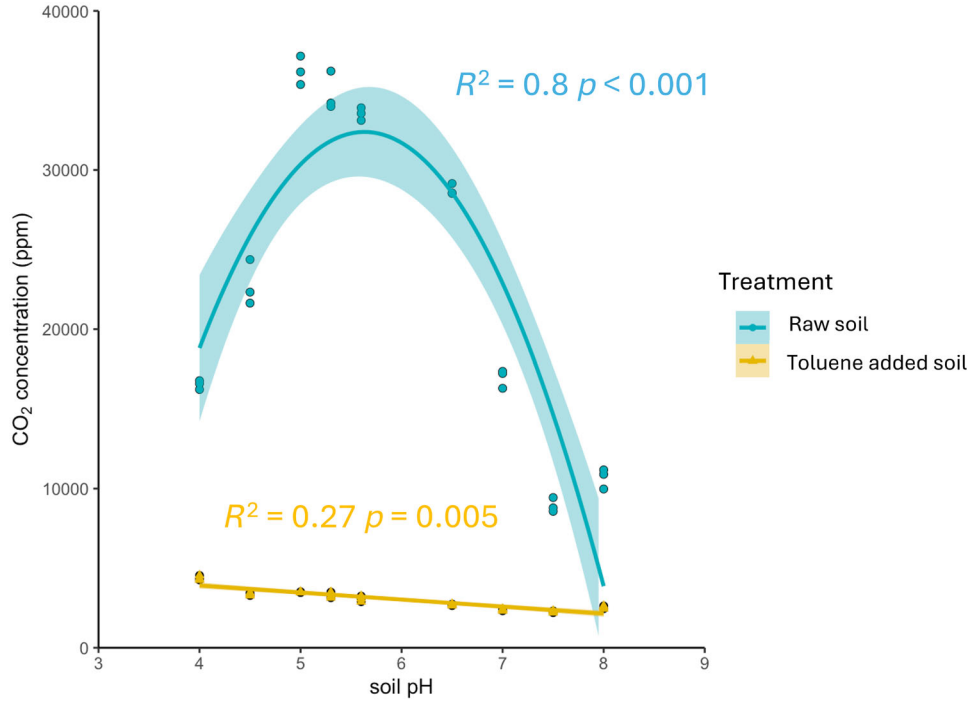

FIGURE 2 Scatter plot of the inhibitory effect of 25% toluene on microbial activity in agricultural soil #105 across a pH range. The blue symbols represent the mean CO_2 respiration of nonsterile soils (±95% confidence intervals [CI], $n = 3$) without toluene addition. The yellow symbols represent the mean CO_2 respiration of soils (±95% CI, $n = 3$) with toluene addition. The coefficients of regression curves were provided using best-fit models.

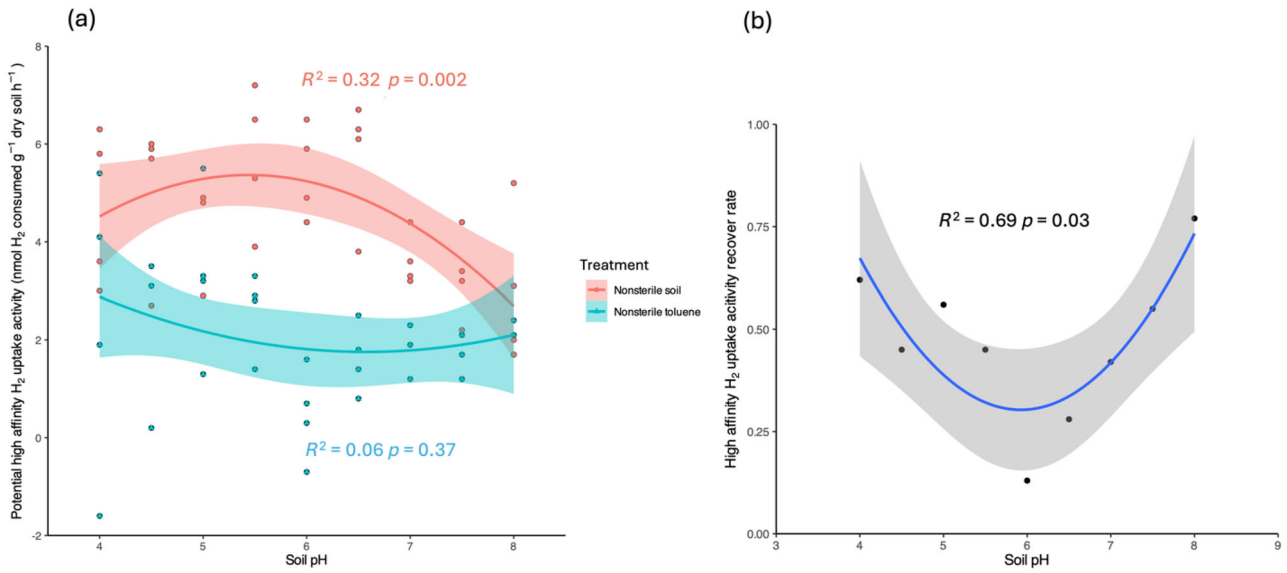

FIGURE 3 The effect of soil pH on biological high-affinity H_2 uptake activity in the presence and absence of 25% toluene. (a) Scatter plot of the toluene effect on high-affinity H_2 uptake activity in agricultural soil #105 with a wide pH range. The red symbols represent the individual H_2 uptake activity of soils without toluene addition. The blue symbols represent the individual H_2 uptake activity of soils with toluene addition. The blue and pink shadows indicate the 95% significant confidence interval ($n = 4$). (b) soil high-affinity H_2 uptake activity recovery rate in toluene-added soil across pH gradient. The recovery rate is defined as the potential high-affinity H_2 uptake activity of soil with toluene addition divided by that without toluene addition. The coefficients of regression curves were provided using best-fit models.

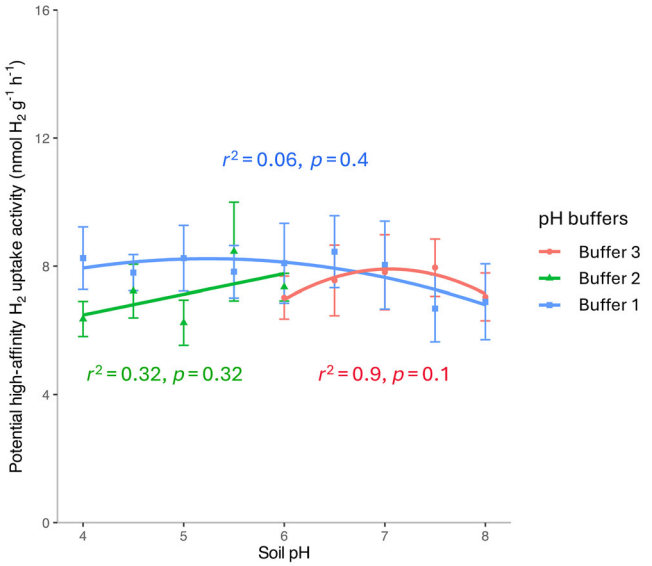

FIGURE 4 The effect of three pH buffer systems on high-affinity H_2 uptake activity in four agricultural soils. The blue symbols represent effects over a wide range of pH using Buffer 1 (citric acid—Na_2HPO_4 buffer); the green symbols show the effects at a range of low pH using Buffer 2 (citric acid—sodium citrate buffer), and the red symbols show effects at a range of high pH using Buffer 3 (Na_2HPO_4—NaH_2PO_4 buffer). Statistical analyses indicated that the three buffer systems had no significant difference ($p = 0.46$). The data are presented in the mean median ± standard errors ($n = 4$). The regression curves were determined using best-fit models.

analysis of variance revealed no effect of the pH buffer system on high-affinity H_2 uptake activity ($p = 0.46$; Table S5), and pH did not influence the high-affinity H_2 uptake activity in the tested agricultural soils ($p = 0.95$; Table S5).

4 | DISCUSSION

4.1 | Selection of the best reference control

Three reference controls were used to assess the soil biological H_2 oxidation. The first control group includes soil amended with 25% toluene (chemical sterilization). This treatment inhibits the activity of intact cellular microbes like other microbial inhibitors, including chloroform and benzene (Yang et al., 2020). We found that toluene addition significantly decreased biological H_2 uptake activity in soil with pH 5–7, with approximately 40%–90% loss, but only 20%–40% of H_2 uptake activity was lost in soil with pH in the acidic and alkaline ranges. Therefore, we cannot use toluene to eliminate microbial-mediated H_2 oxidation activity because of its incomplete inhibition. Furthermore, toluene is not suitable as a reference control. However, according to CO_2 respiration results (Figure 2), the high-affinity H_2 uptake activity was not completely inhibited by toluene compared to the untreated nonsterile soil. High-affinity H_2 uptake activity was inhibited by 70% in acidic and alkaline soils. We suggest that some H_2-oxidized bacteria are toluene-resistant, depending on soil pH, which is consistent with the observation that some H_2-oxidized bacteria are contaminant-resistant (Xu et al., 2020). The H_2-oxidizing bacterial community in soils with diverse pH levels could provide new understanding of the ecological traits of pH-divergent H_2-oxidizing bacteria.

In sterile soil and the sterile buffer controls (heat sterilization), there was no difference in the measured H_2 uptake rate (ppbv min^{-1}) at each pH level ($p = 0.267$; Table S3). This means that microbial activity was completely inhibited by heat sterilization. Sterile soil had a slightly higher H_2 uptake rate than the sterile buffer control, possibly because molecular H_2 is absorbed by clay, binding through Ca ligands, which reduces the gaseous H_2 concentration (Wolff-Boenisch et al., 2023; Zhu et al., 2023). Besides H_2 adsorption, another possibility is that hydroxyl radicals reacted with H_2, thus removing it from the gaseous phase. Overall, the sterile soil was the best reference control for this assay because soil sterilization eliminates the biotic metabolism and without altering the physiochemical properties of the nonsterile soil.

4.2 | Selection of pH buffer system

The high-affinity H_2 uptake activity of agricultural soil was evaluated with three pH buffer systems because each buffer contains salt ions and cations that could affect microbial activity. For example, the citric acid, hydrogen phosphate (HPO_4^{2-}), and dihydrogen phosphate ($H_2PO_4^-$) used in buffer systems can serve as energy sources and be immobilized by microbial biomass (Bhagat et al., 2023). The buffer systems spanned a wide pH range, representing the actual soil pH of agricultural fields in eastern Canada (Canadian Soil Information Service, 2021). None of the tested buffer systems affected the biological H_2 uptake activity significantly (Figures S2 and S3), although the four soils performed differently when exposed to each buffer system. The citric acid—Na_2HPO_4 buffer system is recommended for further research because of its wide pH coverage and buffering capacity, and it apparently did not stimulate or inhibit biological H_2 uptake activity, relative to other buffer systems. We acknowledge that pH buffering does increase the concentration of dissolved ions and other exogenous electron acceptors, providing more substrates for microbial metabolism that may affect the high-affinity H_2 uptake activity (Baril & Constant, 2023). The next logical step in this research is to track the microbial community profile of soils before and after pH buffering. Quantifying the number and type of high-affinity hydrogen oxidizers and hydrogenase classes, by applying metagenomic sequencing of high-affinity H_2 oxidizing marker gene *hhyL* (Giguere et al., 2021), *hucL* (Islam

et al., 2020), group 1f (gene WP_026441619.1, Myers & King, 2016), and group 1l (*HylL*, Ortiz et al., 2020), will be helpful to distinguish how much the H_2 oxidation activity is inhibited by experimental manipulation (e.g., by adding toluene or buffer systems), and how much is due to ecological interaction within soil microbial communities that affect biological H_2 oxidation.

5 | CONCLUSION

The present study describes a method to measure biological H_2 uptake activity by manipulating the soil pH. Heat sterilization of soil is recommended as the reference control. The tested buffer systems had no significant effects on potential high-affinity H_2 uptake activity in soil; however, the citric acid—Na_2HPO_4 buffer system was recommended for further research. Results suggest that toluene-resistant, high-affinity H_2-oxidizing bacteria may exist in acidic and alkaline agricultural soils. Further research is needed to study the impact of different pH environments on the high-affinity H_2-oxidizing bacterial community in soil.

AUTHOR CONTRIBUTIONS

Lijun Hou: Conceptualization; data curation; formal analysis; methodology; validation; visualization; writing—original draft; writing—review and editing. **Philippe Constant**: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing—review and editing. **Joann K. Whalen**: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data presented in this paper are available upon request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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