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Hydrogen oxidation activities in soil as influenced by pH, temperature, moisture, and season

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Summary. Hydrogen oxidation in soil was measured at low (1 ppmv) and high (3000 ppmv) H_2 concentrations to distinguish between the activities of abiontic soil hydrogenases and Knallgas bacteria, respectively. The two activities also showed distinctly different pH optima, temperature optima, and apparent activation energies. The pH optima for the soil hydrogenase activities were similar to the soil pH in situ, i.e., pH 8 in an slightly alkaline garden soil (pH 7.3) and pH 5 in an acidic cambisol (pH 4.6–5.4). Most probable number determinations in the alkaline acidic soils showed that Knallgas bacterial populations grew preferentially in neutral or acidic media, respectively. However, H_2 oxidation activity by Knallgas bacteria in the acidic soil showed two distinct pH optima, one at pH 4 and a second at pH 6.4–7.0. The soil hydrogenase activities exhibited temperature optima at 35–40 °C, whereas the Knallgas bacteria had optima at 50–60 °C. The apparent activation energies of the soil hydrogenases were lower (11–23 kJ mol^{-1}) than those of the Knallgas bacteria (51–145 kJ mol^{-1}). Most of the soil hydrogenase activity was located in the upper 10 cm of the acidic cambisol and changed with season. The seasonal activity changes were correlated with changes in soil moisture and soil pH.

Key words: Soil hydrogenase – Knallgas bacteria – pH optimum – Temperature optimum – Apparent activation energy – Seasonal change

Soils are the most important sink in the atmospheric H_2 budget (Seiler 1978; Conrad 1988). Conrad and Seiler (1980) observed a seasonal change in H_2 deposition in a German soil, with the highest rates during summer. The mechanistic basis and environmental regulation of H_2 oxidation in soil is still largely unknown (reviewed by Conrad 1988). Soil H_2 consumption has been correlated with levels of microbial biomass, ATP, organic C, and moisture in soil (Popelier et al. 1985). Other studies have shown that the uptake of H_2 (or tritium) at low atmospheric concentrations is related to soil temperature, pH, and moisture, and shows characteristic optima (Liebl and Seiler 1976; Conrad and Seiler 1981; Fallon 1982; Förstel 1986). Soils apparently contain two different types of H_2-oxidation activities, which can be distinguished by their K_m values and thresholds for H_2 (Schuler and Conrad 1990). One type of activity is caused by Knallgas bacteria which, however, oxidize H_2 only at relatively high concentrations (>1 ppmv). Atmospheric H_2 concentrations (0.55 ppmv) therefore seem to be exclusively oxidized by abiontic soil hydrogenases (Conrad et al. 1983).

The present study shows that the activities of soil hydrogenases and Knallgas bacteria are distinguished by different pH optima, temperature optima, and activation energies. The potential oxidation of atmospheric H_2 in an acidic cambisol was mainly related to seasonal changes in soil moisture and soil pH.

Materials and methods

Three different soils were used. Soil 1 was an acidic cambisol (sandy clay loam) from the Mainau Forest, Konstanz. The soil had an organic C content of 2.9% and a maximal water-holding capacity of 76.4% (g water 100 g^{-1} dry weight). The soil was repeatedly sampled during the season from the top 10 cm and showed pH values (H_2O) of between 4.6 and 5.4. Soil 2 was a neutral compost soil from the greenhouse of the University of Konstanz (5.7% organic C; 118.3% water-holding capacity; pH 7.0–7.2). Soil 3 was a neutral garden soil from a private garden in Konstanz (5.9% organic C; 85.9% water-holding capacity; pH 7.3–7.4). The soils were passed through a sieve (2 mm) and either used at once or stored at 4 °C in polyethylene bottles.

H_2 oxidation was measured at room temperature (20–25 °C) as described (Conrad and Seiler 1981; Schuler and Conrad 1990). Briefly, the soil samples (100 g) were incubated in glass vessels (1000 ml) under an atmosphere of air containing H_2 at a concentration of either 1 or 3000 ppmv. The H_2 oxidation was determined in gas samples (1–10 ml) which were analyzed for H_2 by gas chromatography with a thermal conductivity detector (Shimadzu, Kyoto, Japan) and an RGD2 Reduction Gas Detector (Techmation, Düsseldorf, Germany). The pseudo-first-order rate constant of H_2 oxidation at 1 ppmv H_2 was deter-

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mined from the logarithmic decrease in the H_2 concentration with time and this value was used to calculate the H_2 oxidation rate by multiplying it with the initial H_2: air ratio (1 ppmv). The H_2 oxidation rate at 3000 ppmv H_2 was determined from the linear decrease in the H_2 concentration with time. To examine the seasonal changes in H_2 activity, samples of soil 1 were taken each month from the same site. One sample was examined immediately; the other sample was dried for 48 h at room temperature and then rehumidified to 25% moisture. The H_2 oxidation activity at 1 ppmv H_2 was measured 2.5 h later. Depth profiles of H_2 oxidation activity were determined by taking several soil cores with a stainless steel corer (1 m length, 3 cm diameter) and pooling the soil from the desired depth. Soil temperature was recorded at the field site with a thermocouple (iron/constantan). Soil moisture was determined gravimetrically after 24 h drying at 104 °C. Soil pH was measured in suspensions of 20 g soil in 20 ml H_2O using a glass electrode.

The effect of soil moisture on H_2 oxidation was assessed using 100 g soil, which was dried at room temperature for 72 h. The soil moisture was adjusted by rehumidification with distilled water. The effect of pH was assessed using 80 g air-dry soil, and the pH was adjusted by spraying the soil with 20 ml HCl or NaOH solutions of different molarities. The pH remained stable during the experiment. The effect of temperature was assessed using 100 g moist (25%) soil, which was incubated in a water bath at temperatures between 5 and 60 °C. H_2 oxidation activity was measured 2.5 h after the temperature adjustment.

The apparent activation energy of H_2 oxidation was determined from plots and linear regression analysis, using the logarithmic form of the Arrhenius equation:

$$\ln v = \ln A - (Ea/R)(1/T)$$

where v is the H_2 oxidation rate; A is the Arrhenius constant; Ea is the apparent activation energy, R is a gas constant, and T is the incubation temperature (K). The regression coefficients ($r > 0.97$) indicated a significant regression of $\ln v$ to $1/T$ at the $P = 1\%$ level.

Knallgas bacteria were counted in the soil samples by the three-tube most probable number technique, using tables from the American Public Health Service (1969). At pH 7.2, the most probable number determinations were done in the mineral medium described by Schlegel et al. (1961), and at pH 5 the determinations were done in the same medium modified to contain 0.5 g Na_2HPO_4 and 1.5 g KH_2PO_4 in 1000 ml H_2O. The most probable number tubes were incubated under an atmosphere of 80% H_2, 10% O_2, 10% CO_2 at 25 °C for 6 weeks to allow chemolithoautotrophic growth of Knallgas bacteria. Positive tubes were assessed by turbidity.

Results and discussion

Seasonal change in potential H_2 oxidation

In the acidic cambisol (soil 1), oxidation of atmospheric H_2 (soil hydrogenase activity) decreased with soil depth. Most of the activity was found in the upper 5–10 cm of the soil (data not shown). Field-fresh samples of the surface layers (10 cm deep) of soil 1 showed the highest activity in August–September, when pH and soil moisture were lowest (Fig. 1). In the wet months (April, June, October), however, when soil moisture and pH were both relatively high, the soil hydrogenase activity was relatively low. The seasonal change in this activity was significantly related to the soil moisture content and the soil pH, but not to seasonal temperature changes (Table 1). Laboratory studies showed that the soil hydrogenase activity was highest at 11% moisture and gradually decreased with an increasing water content. Soil samples that were dried and adjusted to a standard moisture (25%) no longer showed a marked seasonal change in H_2 oxidation (Fig. 1), but still exhibited some correlation ($P > 10\%$) with the soil pH (Table 1). Our results indicate that seasonal changes in H_2 deposition in situ (Conrad and Seiler 1980) may be under the control of soil moisture content and pH rather than soil temperature.

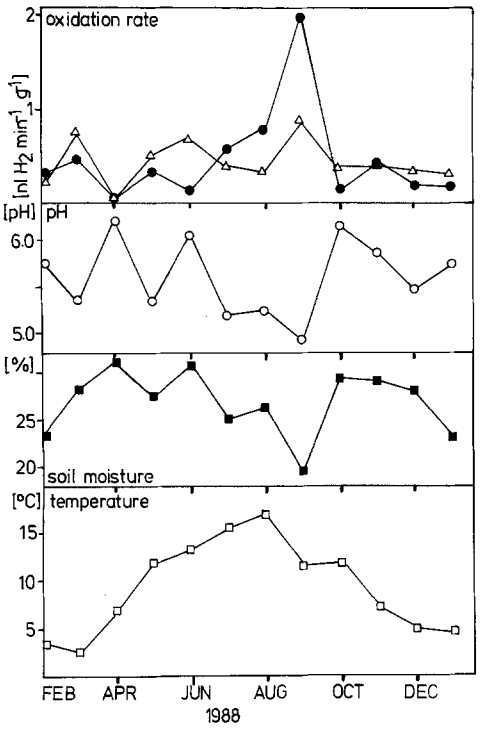

Fig. 1. Seasonal change in H_2 oxidation at low (1 ppmv) H_2 concentrations in an acidic cambisol (soil 1) together with changes in soil pH, soil moisture and soil temperature. ●, Activity at in situ soil moisture; △, activity at 25% soil moisture content

Influence of temperature

The effect of temperature on H_2 oxidation by soil hydrogenase activity and by Knallgas bacteria was studied in the acidic cambisol (soil 1) and the neutral compost soil (soil 2). Increasing temperatures in general increased the H_2 oxidation activity until an optimum temperature was reached. Beyond this temperature the activity decreased again. The temperature optima and apparent activation energies differed from soil 1 to soil 2, and were also different at the optimum soil hydrogenase and Knallgas bacteria activities, measured at low (1 ppmv) and high (3000 ppmv) H_2 concentrations, respectively. The soil hydrogenases in soil 1 (Fig. 2a) and soil 2 (Fig. 2b) each had a single optimum temperature, 40 and

Table 1. Correlation matrix of potential H_2 oxidation activity at low H_2 concentrations and of soil variables subject to seasonal changes

Soil parameter	H_2 oxidation activity at	
	In situ moisture	Standard moisture
Moisture	−0.722**	−0.281
pH	−0.756***	−0.447*
Temperature	0.341	0.135

*$P < 0.1$, **$P < 0.01$, ***$P < 0.005$. Soil moisture was adjusted to 25% H_2O

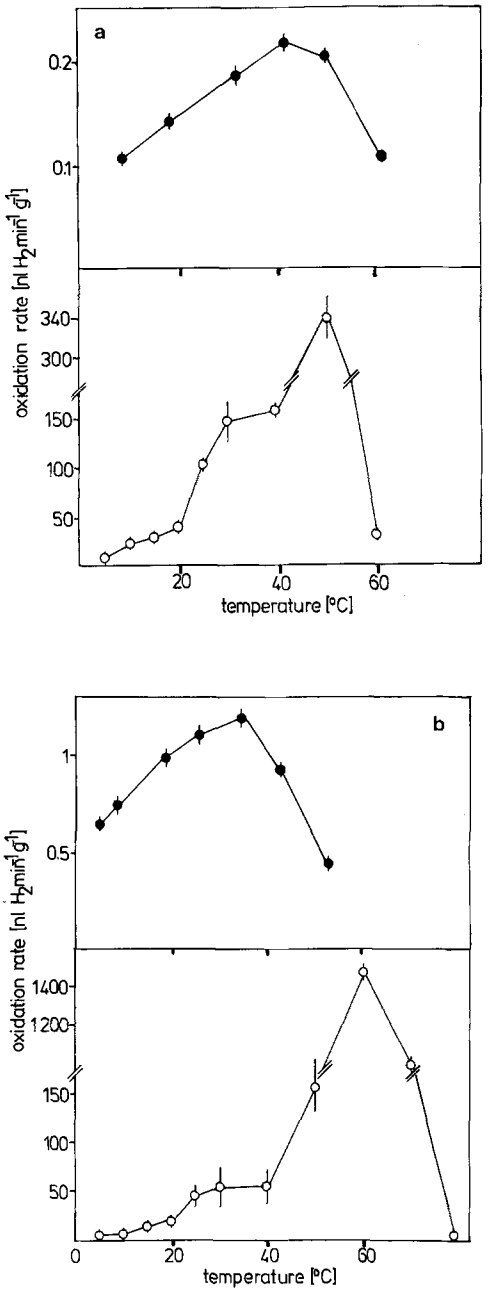

Fig. 2a, b. Influence of temperature on H_2 oxidation in **a** an acidic cambisol and **b** a compost soil. The activity was measured at low (1 ppmv; ●) and high (3000 ppmv; ○) H_2 concentrations

35 °C, and a single activation energy, 23 and 11 kJ mol^{-1}, respectively. These relatively low apparent activation energies were comparable to those calculated using H_2 uptake data from Liebl and Seiler (1976) and using tritium uptake data from Fallon (1982) and Förstel (1986), giving values of 13, 39 and 5 kJ mol^{-1}, respectively. The reason why soil hydrogenases have such low activation energies is not known. However, these low activation energies do explain the poor correlation between soil temperature and the rate of oxidation of atmospheric H_2 during field campaigns (Liebl and Seiler 1976; Conrad and Seiler 1985).

Table 2. Numbers of Knallgas bacteria in soil determined in media with an acidic or a neutral pH

	pH 5	pH 7.2
Soil 1	4.6×10^4	4.6×10^3
Soil 3	1.1×10^4	4.6×10^5

Three-tube most probable number counts; 95% confidence interval about ±85%

Knallgas bacteria had generally higher activation energies (51–145 kJ mol^{-1}). Soil 1 showed a shoulder at 30–40 °C (Fig. 2a) in addition to a maximum at 50 °C, but showed only a single activation energy (51 kJ mol^{-1}) in the Arrhenius plot. In contrast, soil 2 had two temperature optima, one between 25 and 40 °C and the other at 60 °C (Fig. 2b). These two optima were also differentiated by the Arrhenius plot (data not shown), which gave activation energies of 95 kJ mol^{-1} and 145 kJ mol^{-1}, respectively. These results suggest the presence of two microbial populations with different temperature characteristics. The high temperature maxima indicate the presence of thermophilic Knallgas bacteria; this is surprising since the soils had not been exposed to high temperatures in recent times. While the compost soil may have experienced high temperatures during the composting process (Farquhar and Rovers 1973), the highest annual temperature to which the acidic cambisol was exposed was less than 20 °C.

Influence of pH

The effect of pH on H_2 oxidation by soil hydrogenase and Knallgas bacteria was studied in the acidic cambisol (soil 1) and the slightly alkaline garden soil (soil 3). Soil 3 was chosen because the pH of soil 2 could not be adjusted to stable values. Soil hydrogenase activity was strongly influenced by soil pH. The activity in soil 1 showed an optimum at pH 5 (Fig. 3a). This pH optimum was close to the acidic in situ pH (4.6–5.4) of soil 1. A second pH optimum was visible only as a slight bump (one data point) at pH 8 and thus is debatable. Soil 3 (pH 7.3–7.4), however, showed optimum activity at an alkaline pH (pH 8) (Fig. 3b). Unfortunately, the pH in soil 3 could not be adjusted to stable values at pH <6 and therefore could not be assayed at low pH. Our results show, however, that the optimum pH for the soil hydrogenase activity was in the same range as the in situ pH.

H_2 oxidation by Knallgas bacteria was also strongly influenced by soil pH. However, in the acidic soil 1 this activity showed two distinct pH optima, one at pH 4 and another at pH 6.4–7.0 (Fig. 3a). In contrast the slightly alkaline soil 3 had one optimum at pH 7.3 (Fig. 3b). The most probable number count of Knallgas bacteria in the acidic soil 1 were 10 times higher at pH 5 than at pH 7.2 (Table 2). In soil 3, however, the bacterial counts were about 40 times higher at pH 7.2. Our results show that the Knallgas bacteria were, like the soil hydrogenases, adapted to the in situ soil pH, i.e., slightly alkaline in the

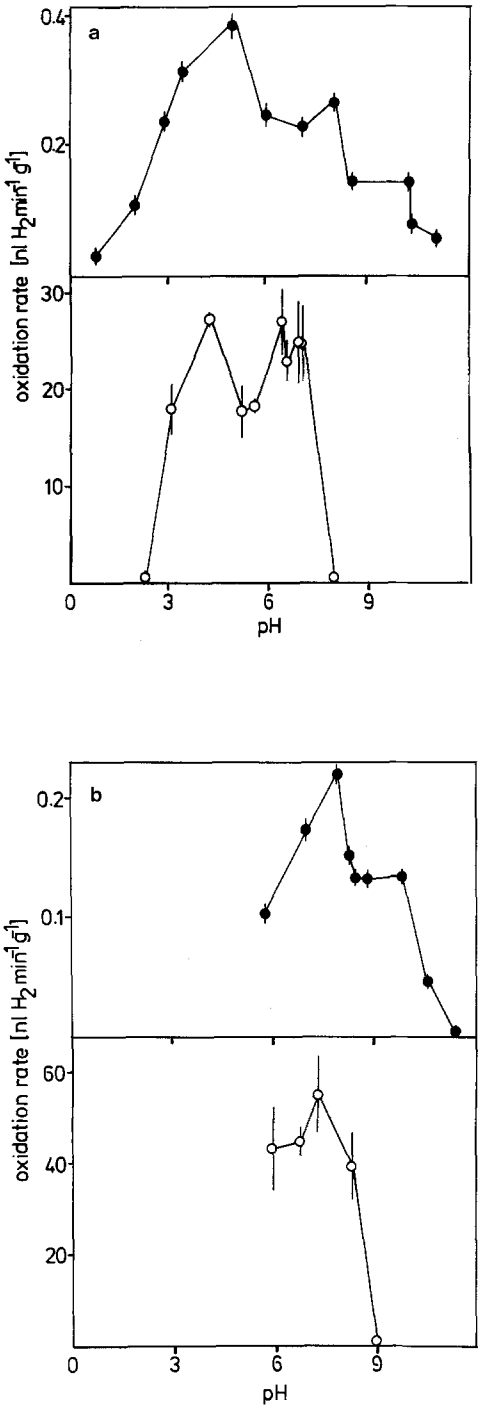

Fig. 3. a, b. Influence of soil pH on H_2 oxidation in **a** an acidic cambisol and **b** a garden soil. The activity was measured at low (1 ppmv; ●) and high (3000 ppmv; ○) H_2 concentrations

garden soil and acidic in the cambisol. However, the acidic cambisol apparently had a second bacterial population with a pH optimum around neutrality, and the neutral H_2 oxidation activity was as high as the acidic one. The Knallgas bacteria were counted in media with an acidic and with a neutral pH. However, the numbers were about 10 times higher at an acidic pH. This observation suggests that acidophilic Knallgas bacteria may be common in acidic soils.

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