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Effect of methane on soil dehydrogenase activity

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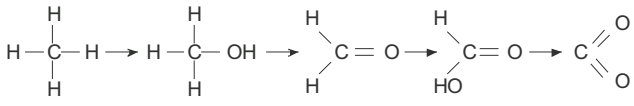
A b s t r a c t. Changes in soil respiration and dehydrogenase activity as effected by methane oxidation were studied in two loess soils under laboratory conditions. Stimulation of soil dehydrogenases reached the maximum at the beginning of the rapid CH_4 and O_2 depletion and intensive CO_2 production. After 7-day incubation with CH_4, dehydrogenase activity increased by 112 and 66% in an Eutric Cambisol and a Haplic Phaeozem, respectively ($P<0.001$). The Phaeozem respired more intensively than Cambisol, but the stimulation of the respiration by CH_4 supply was evidently higher in the Cambisol. The methanotrophic activity of the soils tested varied in their relation to the respiration. The molar ratios of CH_4 oxidized to O_2 consumed to CO_2 evolved was as 1 mol:1.44 mol:0.52 mol in Cambisol, and 1 mol:1.03 mol:0.24 mol in Phaeozem.

K e y w o r d s: soil, dehydrogenase activity, methane oxidation

INTRODUCTION

Understanding of mechanisms regulating methane oxidation in soil is important because of the continuous increasing CH_4 concentration in the atmosphere (Stępniewski and Pawłowska, 1996). Soil methanotrophic activity results from the natural ability of soil microorganisms to utilize CH_4 as a carbon and energy source. Four stages in the oxidation of methane are distinguished (Murell, 1992):



The first reaction, catalyzed by methane monooxygenase enzyme, is followed by the stages with actions of methanol dehydrogenase, formaldehyde dehydrogenase and formate-dehydrogenase, respectively. Assays for dehydrogenase activity in soil have often been used to achieve an index of the total soil microbial activity. The test involves the activity of numerous intracellular enzymes (dehydrogenases) taking part in energetic metabolism of all microbial cells. Soil capacity for methane oxidation is frequently studied in order to elucidate its dependence on fertilization (mineral and organic), environmental conditions, soil management, *etc*. (Bradford *et al*., 2001; Hütsch, 1998; Stępniewski and Zygmunt, 2000).

The aim of the study was to determine the changes in soil dehydrogenase activity during methane oxidation in two loess soils. The study was performed under laboratory conditions to eliminate the effect of soil temperature and moisture.

MATERIALS AND METHODS

Two soils developed from loess were studied, an Eutric Cambisol (Bli) and a Haplic Phaeozem (Cli), exhibiting similar granulometric composition and pH (Table 1). The soils were selected from the collection of the Bank of Soil Samples (Gliński *et al*., 1991).

Soil samples (3 g air-dry mass) were incubated in 25.6 cm^3 glass flasks at 25°C in the dark. They were moistened to the level of soil water potential of -159 hPa (pF 2.2). These conditions were selected to ensure suitable humidity and aeration status (Gliński and Stępniewski, 1985; Witkowska-Walczak *et al*., 2003). Soil water content was 26.8 and 30.6% g g^{-1} for Cambisol and Phaeozel, respectively. Soil redox potential (Eh) was monitored during the 11-day experiment to be >450 mV, indicating that sufficient oxygen was available.

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T a b l e 1. Basic characteristics of the soils studied

Soil	Location	Depth (cm)	Organic matter (%)	pH_{H2O}	Granulometric composition (%) (dia in mm)		
					2-0.1	0.1-0.002	<0.002
Eutric Cambisol	Zamość	0-20	0.95	7.71	5	83	12
Haplic Phaeozem	Opole	0-50	1.21	7.19	4	90	6

Methane was injected through rubber septa to obtain 5% (v/v) CH_4 concentration in the soil headspace. Parallel control soils were incubated without methane. An additional set of flasks was prepared for each soil: three replications for each treatment were opened on successive days to measure redox potential and to assay soil dehydrogenase activity. Changes in gas concentrations (CH_4, CO_2 and O_2) were measured by gas chromatographic methods (Włodarczyk, 2000). Soil dehydrogenase activity was determined by the method with TTC (2,3,5-triphenyl tetrazolium chloride) according to Casida *et al*. (1964).

RESULTS AND DISCUSSION

Both soils exhibited similar dynamics of methanotrophic activity (Fig. 1). A relatively long lag-phase was observed with slight changes in CH_4 concentration during initial 6 days of incubation (Fig. 1a). Then, between 7 and 11th day, methane disappeared rapidly in both soils. Simultaneously, during the same period (7-11th day), a distinct alteration in concentrations of both CO_2 and O_2 was observed in CH_4-amended soils. The control (not amended) soils, however, continued slight changes, as in the earlier period (Fig. 1b-c). The soils showed different levels of respiration activity. Eutric Cambisol evolved 320 mg CO_2–C kg^{-1}, and consumed 8% vol. O_2, whereas Haplic Phaeozem evolved 403 mg CO_2-C kg^{-1} and utilized 11% O_2 vol. during the 11 day incubation. Methane addition resulted in an enhancement of CO_2 production by 138 mg CO_2-C kg^{-1} and 65 mg CO_2-C kg^{-1} (in Bli and Cli, respectively) as well as of oxygen consumption by 8.9% and 6.5% vol., respectively (Table 2). Thus, the average net cost of the oxidation of added CH_4 was 85% O_2 and 30% CO_2 (setting the gas exchange in the controls to be 100%).

A simple equation for the methanotrophic activity can be adopted (Large, 1983):

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O.$$

According to this equation, the complete oxidation of 1 mol CH_4 is accompanied by the utilization of 2 mol O_2 and production of 1 mol CO_2. However, this simplified assumption does not include the link of methane oxidation with other metabolic pathways, *eg* assimilation of CH_4-derived carbon into microbial biomass, leading to the shift in relations of exchanged gases. Based on the

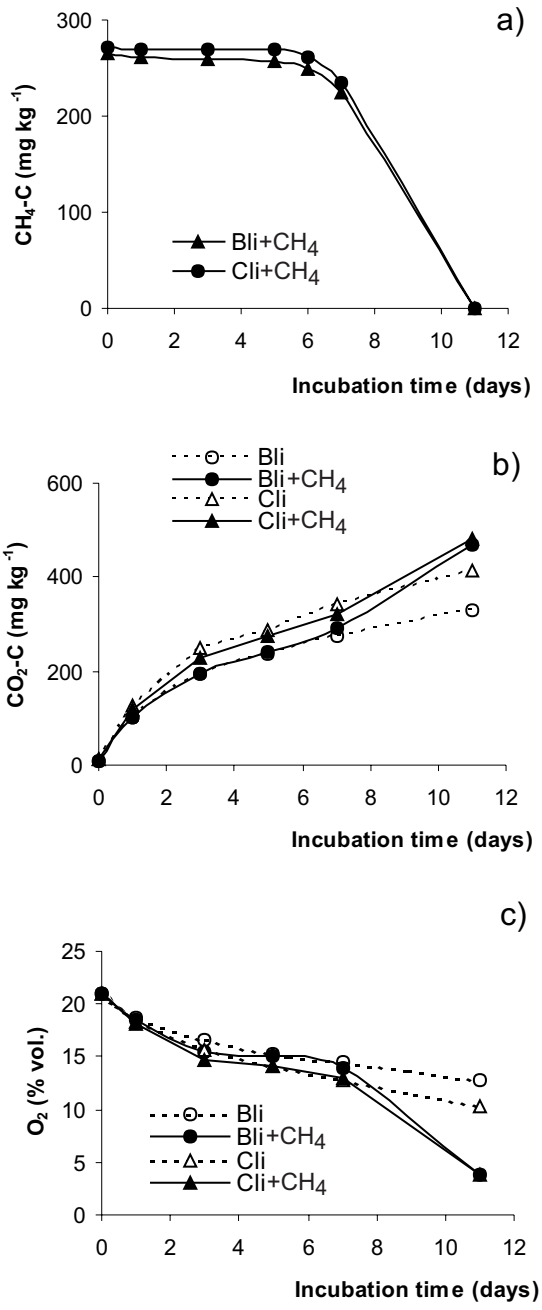

Fig. 1. Changes of CH_4 (a), CO_2 (b) and O_2 (c) in the headspace of loess soils incubated without methane (discontinuous lines) or with methane (+CH_4, continuous lines). Bli – Eutric Cambisol, Cli – Haplic Phaeozem.

T a b l e 2. Comparison of the total amounts of CO_2 produced and O_2 consumed during 11-day incubation of two mineral soils with and without methane

Soil variant	Methane oxidized (mg CH_4-C kg^{-1})	CO_2 evolved		O_2 utilized	
		(mg CO_2-C kg^{-1})	% Ctrl[a]	(% vol.)	% Ctrl
Bli (Control)	0	320	100	8.2	100
Bli + CH_4	265	458	143	17.1	209
Cli (Control)	0	403	100	10.7	100
Cli + CH_4	271	467	116	17.2	161

[a]% Ctrl – percent in relation to control soil not amended with methane.

experiment, two different ratios for the tested soils were calculated:

1 mol CH_4 : 1.44 mol O_2 : 0.52 mol CO_2 (Bli)

1 mol CH_4 : 1.03 mol O_2 : 0.24 mol CO_2 (Cli).

The results are in agreement with the ranges noted by other authors (0.2-1.8 mol O_2 per mol CH_4, and 0.2-0.9 mol CO_2 per mol CH_4) (Hoeks, 1972; Stępniewski and Pawłowska, 1996).

Phaeozem showed nearly 3 times higher level of dehydrogenase activity as compared with Cambisol at the start of the experiment (Fig. 2). However, after an initial decrease in enzyme activity observed for both variants (with and without CH_4 addition), this difference between both soils was much lower. The lowering of the enzyme activity resulted presumably from a depletion of the easily available, native substrate due to its rapid consumption by microbial populations, intensively developing in the rewetted soil (stored previously as air-dry). In the control - not amended - soils, the dehydrogenase activity continued to decrease till the end of incubation (Fig. 2). Nevertheless, an increase in dehydrogenase activity in CH_4-amended soils was observed on the 7th day.

The average dehydrogenase activity in the control and amended Bli soils were 0.0330 and 0.0357 nmol TPF $g^{-1}min^{-1}$, respectively, whereas in the more active Cli soil, values of 0.0740 and 0.0878 nmol TPF $g^{-1}min^{-1}$, respectively, were observed. Thus, methane stimulated dehydrogenases by 8 and 19% in Bli and Cli soils, respectively (mean values for the entire incubation period).

The DHA-differences (being a direct measure of dehydrogenase stimulation by methane) were the highest on the 7th incubation day ($P<0.001$) for both soils (Fig. 3), and occurred simultaneously with the beginning of a rapid methane and oxygen depletion as well as accelerated carbon dioxide emission (Fig. 1b,c). Dehydrogenase activity of the CH_4-amended variants was, at that moment, higher by 112 and by 66% (in Bli and Cli, respectively) than those of controls. The strong stimulation of dehydrogenase was preceded by a period of adaptation of soil methanotrophic microorganisms, which was characterized by relatively low ΔDHA values as well as slight changes in methane concentration. CH_4-dependent increase in dehydrogenase activity was great as compared to the maximum increase of 15% which was previously observed for CH_4-amended Mollic Gleysol (Brzezińska *et al*., 1998).

The modification of the soil biological activity, being a response to CH_4 utilization, depends on the properties of the soil system *eg* microbial populations and nutrients availability. The supply of CH_4 resulted in an increase of both respiration (as measured by O_2 uptake and CO_2

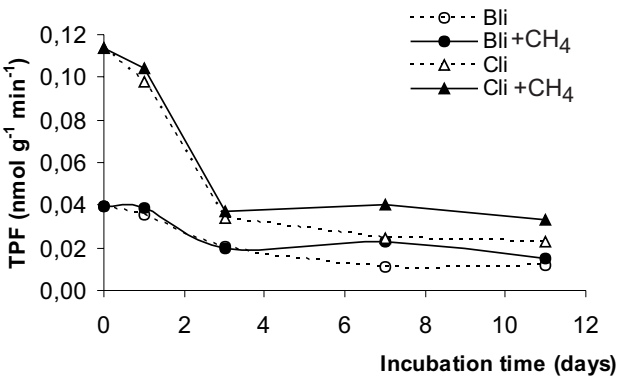

Fig. 2. Dehydrogenase activity in soil incubated with or without methane. Explanations as in Fig. 1.

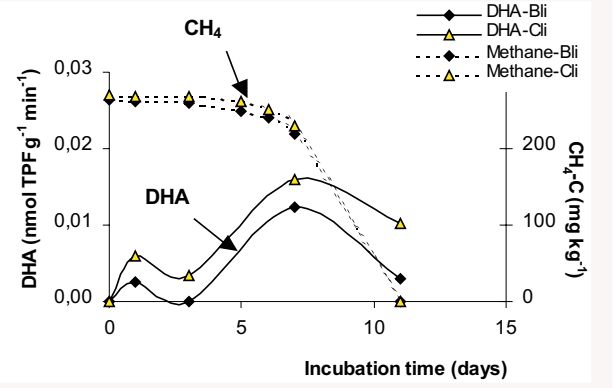

Fig. 3. The effect of CH_4 on soil dehydrogenase activity. ΔDHA – difference between dehydrogenase activity in methane-amended and control soils ($\Delta DHA = DHA_{CH_4} - DHA_{control}$).

production) and dehydrogenase activity. This stimulation was even higher in the soil which showed lower activity when incubated without methane amendment (Eutric Cambisol) than in the soil with higher 'native' activity (Haplic Phaeozem).

CONCLUSIONS

1. Methane addition significantly affected dehydrogenase activity in the loess soils tested.

2. Dehydrogenase activity in a CH_4-amended Eutric Cambisol and a Haplic Phaeozem was higher, by a maximum of 112 and 60%, respectively, than that activity in not amended-control soils ($P<0.001$).

3. The highest stimulation of soil dehydrogenase occurred in the period of rapid depletion of CH_4 and O_2 and simultaneous intensive CO_2 production.

4. O_2 consumption and CO_2 production were, on average, 85 and 30% higher, respectively, in CH_4-amended soils as compared to the controls.

5. Gas exchange, related to the methanotrophic activity, was in the ratios of 1 mol CH_4 : 1.03-1.44 mol O_2 : 0.24-0.52 mol CO_2.

REFERENCES

Bradford M.A., Ineson P., Wookey P.A., and Lappin-Scott H.M., 2001. Role of CH_4 oxidation, production and transport in forest soil CH_4 flux. Soil Biol. Biochem., 33, 1625-1631.

Brzezińska M., Kuzyakov Y., Włodarczyk T., Stahr K., and Stępniewski W., 1998. Oxidation of methane and dehydrogenase activity in a Mollic Gleysol. Z. Pflanz. Bodenk., 161, 697-698.

Casida L.E.JR., Klein D.A., and Santoro T., 1964. Soil dehydrogenase activity. Soil Sci., 98, 371-376.

Gliński J., Ostrowski J., Stępniewska Z., and Stępniewski W., 1991. Soil sample bank representing mineral soils of Poland (in Polish). Problemy Agrofizyki, 66, 4-57.

Gliński J. and Stępniewski W., 1985. Soil Aeration and Its Role for Plants. CRC Press. Boca Raton, Florida, 39-89.

Hoeks J.U., 1972. Effect of leaking natural gas on soil and vegetation in urban areas. Agricultural Research Reports 778, Wageningen, Netherlands, 27-59.

Hütsch B.W., 1998. Methane oxidation in arable soil as inhibited by ammonium, nitrite, and organic manure with respect to soil pH. Biol. Fertil. Soils, 28, 27-35.

Large P.J., 1983. Methylotrophy and Methanogenesis. Aspects of Microbiology 8. American Society for Microbiology. Van Nostrand Reinhold, Wokingham, UK.

Murell J.C., 1992. Genetics and molecular biology of methanotrophs. FEMS Microbiology Reviews, 88, 233-248.

Stępniewski W. and Pawłowska M., 1996. A possibility to reduce methane emission from landfills by its oxidation in the soil cover. In: Chemistry for the Protection of the Environment (Eds L. Pawłowski, W.J. Lacy, Ch.G. Uchrin, M.R. Dudzińska). Plenum Press, New York, 75-92.

Stępniewski W. and Zygmunt M., 2000. Methane oxidation in homogenous soil covers of landfills: a finite – element analysis of the influence of gas diffusion coefficient. Int. Agrophysics, 14, 449-456.

Witkowska-Walczak B., Walczak R., and Ostrowski J., 2003. Pore size distribution and amount of water available for plants in arable soils of Poland. Int. Agrophysics, 17, 213-217.

Włodarczyk T., 2000. N_2O emission and absorption against a background of CO_2 in Eutric Cambisol under different oxidation-reduction conditions (in Polish). Acta Agrophysica, 28, 5-132.