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Influence of methane concentration on methanotrophic activity of Mollic Gleysol and Haplic Podzol

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A b s t r a c t. The influence of methane concentration on the methanotrophic and respiration activity of two soils (Mollic Gleysol and Haplic Podzol), characterized by similar granulometric composition and pH values but different organic matter content, was studied in a model experiment. The two investigated soils showed significant differences in the daily methane consumption – 48.2 mg CH_4-C kg^{-1} d^{-1} for Mollic Gleysol and 5.95 mg CH_4-C kg^{-1} d^{-1} for Haplic Podzol, as well as in the daily oxygen consumption – 0.88% and 0.33% O_2 d^{-1} for Mollic Gleysol and Haplic Podzol, respectively. The soils studied exhibited high (Mollic Gleysol) and low (Haplic Podzol) affinity methane oxidation. Daily CO_2 production increased with increasing methane amendment and ranged from 10.6 to 87.7 ml CO_2 kg^{-1} d^{-1} and was about 2.5 fold higher in the Mollic Gleysol than in the Haplic Podzol. The investigated soils showed different values of the CH_4:O_2:CO_2 ratio.

K e y w o r d s: methane concentration, methanotrophic activity, respiration

INTRODUCTION

Methane is the main hydrocarbon present in the atmosphere, with an average concentration of 1.7 ppm (Dlugokencky *et al*., 1994). The atmospheric concentration of CH_4, which is a greenhouse gas, has increased and more than doubled during the past 200 years (Mancinelli, 1995). Methane sources are mainly wetlands. However, 60% to more than 90% of CH_4 produced in the anaerobic zones of wetlands is deoxidised in their aerobic zones. Methane consumption occurs in most soils and exhibits a broad range of values. Highest consumption rates or potentials are observed in soils where methanogenesis is or has been effective and where CH_4 concentration is or has been much higher than in the atmosphere (Le Mer and Roger, 2001). Identification of the factors influencing the flux of methane into the atmosphere is becoming increasingly important.

We studied the influence of methane concentration on the methanotrophic and respiration activity of Mollic Gleysol and Haplic Podzol, characterized the similar granulometric composition and pH values but different organic matter content, in a model experiment.

MATERIALS AND METHODS

Soil samples (Mollic Gleysol and Haplic Podzol - A_p horizons) were taken from the Bank of Polish Mineral Soils of the Institute of Agrophysics PAS, Lublin. The main characteristics of the soils investigated are shown in Table 1.

Air-dry soils, stored for several months at ambient temperature, were sieved through a 1 mm sieve. Soil moisture was adjusted to water tension of 159 hPa (pF 2.2). The soil samples (corresponding to 6 g of air-dry mass) were placed in 60 cm^3 glass vessels (standard units) and tightly sealed with rubber stoppers. Methane was added to the closed vessels in the following concentrations: 1.25, 2.5, 5, 10 and 15%, and additionally 0.5 and 0.7% to Mollic Gleysol. The vessels without methane addition were used as control. The soils were incubated at 20 C in O_2 atmosphere and monitored for CH_4, CO_2 and O_2 after 1, 3, 7, 9, 10, 11, 14, 16, 20, 22, 27 and 28 days by sampling the headspace (up to the end of methane oxidation). The CH_4, CO_2 and O_2 were determined gas chromatography using a Shimadzu GC-14 (Japan) fitted with a thermal conductivity detector at 60^{o}C. Gas samples were analyzed at 40 C with the use of two 2 m columns, one packed with a Porapak Q and the second with a molecular sieve 5A with He as a carrier gas

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T a b l e 1. Basic characteristic of investigated soils

Soil	Organic matter (%)	pH	Granulometric composition (%) (*dia* in mm)		
			1-0.1	01-0.002	<0.002
Mollic Gleysol	3.69	7.3	59	40	1
Haplic Podzol	0.76	6.9	50	48	2

flowing at a rate of 40 ml min^{-1}. The concentrations of CH_4, CO_2 and O_2 were corrected for solubility in water by using published values of the Bunsen absorption coefficient (Gliński and Stępniewski, 1985). Incubation vessels were replicated three times. The $CH_4 : O_2 : CO_2$ ratio was calculated for the O_2 consumption and CO_2 production as a difference between CH_4-amended soil minus the control without CH_4 amendment.

RESULTS AND DISCUSSION

Figure 1 shows CH_4-C concentration in the soil head-space of Mollic Gleysol and Haplic Podzol as a function of incubation time. The data showed different courses of methane oxidation. At the beginning of incubation, a slight methane oxidation was observed, probably related with the adaptation of methanotrophs to the experimental conditions. This period lasted 3 days for Mollic Gleysol for all methane amendments and 7 days for Haplic Podzol in the case of higher methane addition (5, 10 and 15%), and 11 days in the case of lower CH_4 concentration. After this period, rapid oxidation of methane occurred in Mollic Gleysol during the next 3 days, when CH_4 content dropped to 0 with the exception of 15% CH_4 (Fig. 1a). A slightly different course of methane consumption was observed in Haplic Podzol, where CH_4 oxidation was slower than in Mollic Gleysol and depended on methane concentration (Fig. 1b). Intensive CH_4 sink in this soil occurred during its incubation between 7 and 22 days with 5, 10 and 15% methane additions, whereas methane oxidation with 1.25 and 2.5% CH_4 additions was lower. In no case CH_4 content dropped to 0.

Methanotrophic activity of investigated soils as dependent on CH_4 amendment, expressed as daily oxidation is shown in Fig. 2. Much higher methane oxidation was observed in Mollic Gleysol (Fig. 2a) than in Haplic Podzol (Fig. 2b). Methanotrophic activity increased with increasing initial CH_4 concentration in the soil and ranged from 2.24 to 134.8 mg CH_4 -C kg^{-1} d^{-1} for Mollic Gleysol and only from 0.79 to 19.1 CH_4-C kg^{-1} d^{-1} for Haplic Podzol. The lower methanotrophic activity of Mollic Gleysol with the addition of 15% of methane (61.8 mg CH_4-C kg^{-1} d^{-1}) should be emphasized. It can be seen that in this case the effect of inhibition by the substrate surplus was observed.

The soils investigated differed with regard to methane oxidation, which was confirmed by variation analysis (Fig. 3). The average daily methane oxidation for Mollic Gleysol was 48.2 mg CH_4-C kg^{-1} d^{-1} and only 6 mg CH_4-C kg^{-1} d^{-1} for Haplic Podzol.

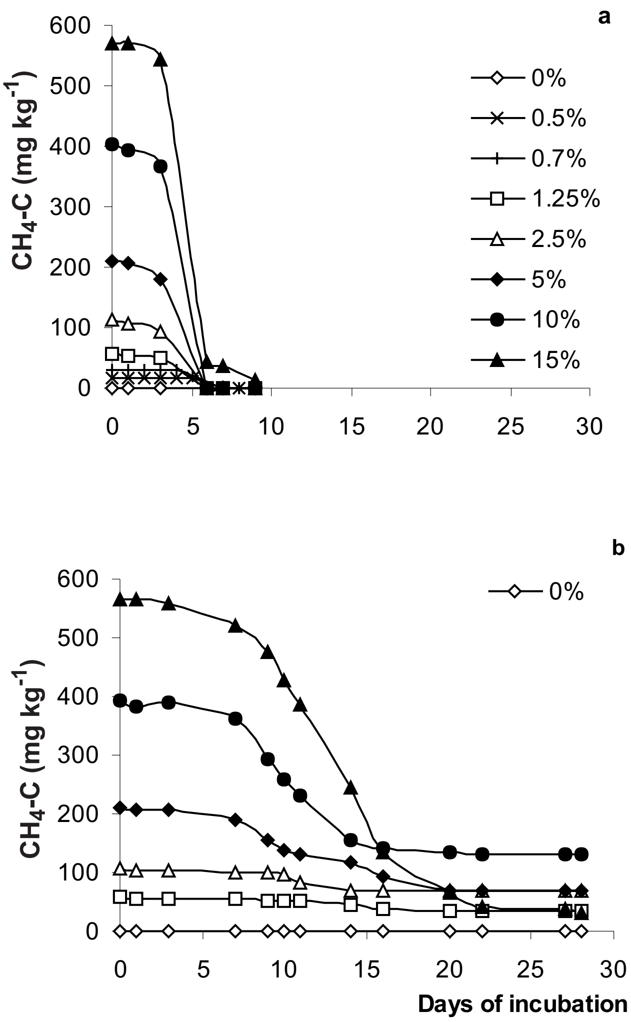

Fig. 1. The course of methane content (CH_4-C mg kg^{-1}) in the headspace during the incubation of the Mollic Gleysol (a) and the Haplic Podzol (b).

The results presented showed that methanotroph occurrence in the investigated soils was characterized by differing requirements with respect to methane concentration. Methanotrophs existing in Mollic Gleysol

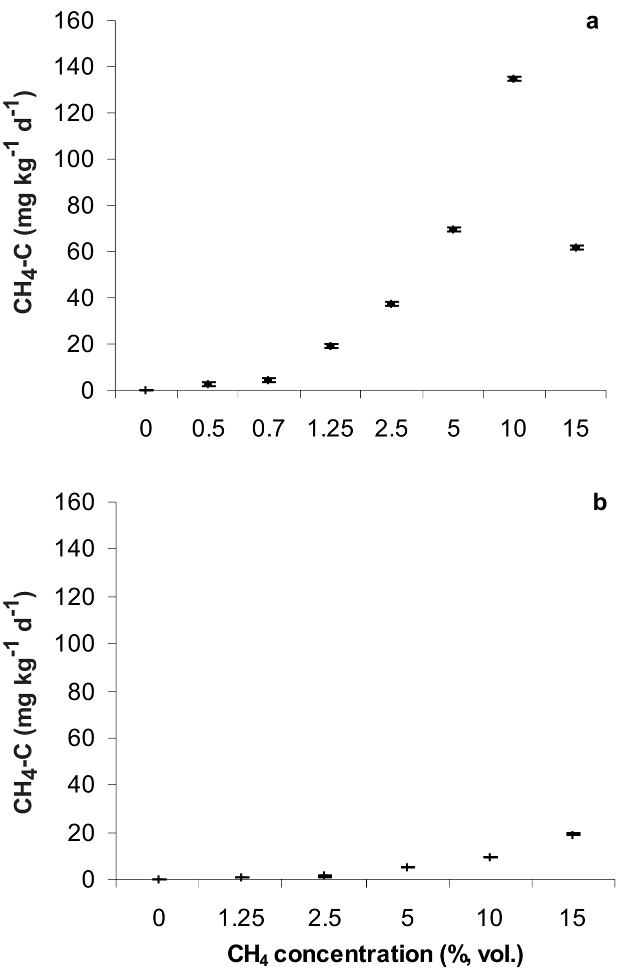

Fig. 2. Daily methane oxidation (CH_4-C mg kg^{-1} d^{-1}) in the Mollic Gleysol (a) and the Haplic Podzol (b) as a function of CH_4 concentration. The bars represent 95% LSD confidence intervals.

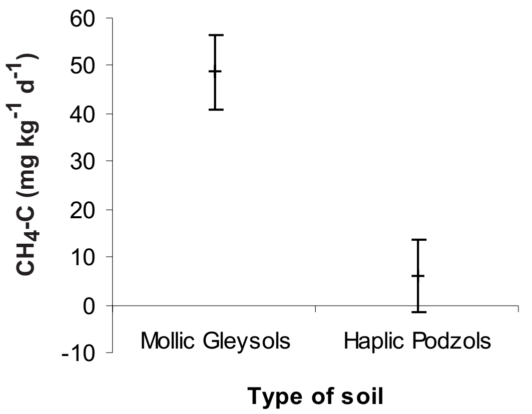

Fig. 3. Daily methane oxidation (CH_4-C mg kg^{-1} d^{-1}) as a function of the type of soil. The bars represent 95% LSD confidence intervals.

were adapted to oxidation of low methane concentration, which characterizes high affinity oxidation (defined as the reciprocal of Michaelis constant, K_M). These were probably facultative methanotrophs. Methanotrophs inhabiting Haplic Podzol needed higher methane concentration and were responsible for low affinity oxidation. These were probably obligatory methanotrophs which use CH_4 as only a C and energy source. Two forms of CH_4 oxidation are recognized in soils (Bender and Conrad, 1992; 1993). The first form, known as 'high affinity oxidation', occurs at CH_4 concentration close to that of the atmosphere - <12 ppm (Topp and Hanson, 1993). The second form, known as 'low affinity oxidation', occurs at CH_4 concentration higher than 40 ppm. It is performed by bacteria called methanotrophs and is considered to be the true methanotrophic activity (King *et al*., 1990; Whalen *et al*., 1990). In the case of Mollic Gleysol, bacterial population responsible for high affinity oxidation utilized completely 17.9 ppm of CH_4 added (well below 40 ppm), whereas Haplic Podzol methanotrophs needed much higher methane concentration than 40 ppm for more efficiency of CH_4 oxidation (Fig. 2b).

Figure 4 shows O_2 concentration in the soil headspace of Mollic Gleysol and Haplic Podzol as a function of incubation time. The data shows different courses of oxygen consumption. At the start of incubation (during the first two days) a slight oxygen disappearance, similar to methane oxidation, was observed, independently from methane concentration. After introductory methanotroph adaptation to the experimental conditions, a distinct drop of oxygen concentration, proportional to CH_4 addition, was noticed. The highest O_2 consumption was observed between 3rd and 6th days of incubation in Mollic Gleysol (Fig. 4a) and between 7th and 16th days in the case of Haplic Podzol (Fig. 4b). This period coincided with the highest methane oxidation very well.

Figure 5 shows daily O_2 consumption with reference to initial CH_4 concentration. Daily oxygen concentration disappearance increased with increasing methane amendment and was proportional to CH_4 oxidation. It should be emphasised that a distinct difference in the respiration activity of the investigated soils was caused by methane addition because their respiration activity without methane activity was comparable and equal to 0.2 and 0.12% O_2 d^{-1} for Mollic Gleysol and Haplic Podzol, respectively. It seems that native organic matter content differentiated the metabolism of microorganisms inhabiting the soils under study, among others with respect to ability of methane oxidation. Much higher daily oxygen disappearance was observed in Mollic Gleysol (Fig. 5a) than in Haplic Podzol (Fig. 5b). Daily oxygen consumption increased with increasing CH_4 concentration in the soils and ranged from 0.36 to 1.82% O_2 d^{-1} for Mollic Gleysol and from 0.15 to 0.66% O_2 d^{-1} for Haplic Podzol. The average daily oxygen consumption by Mollic Gleysol was

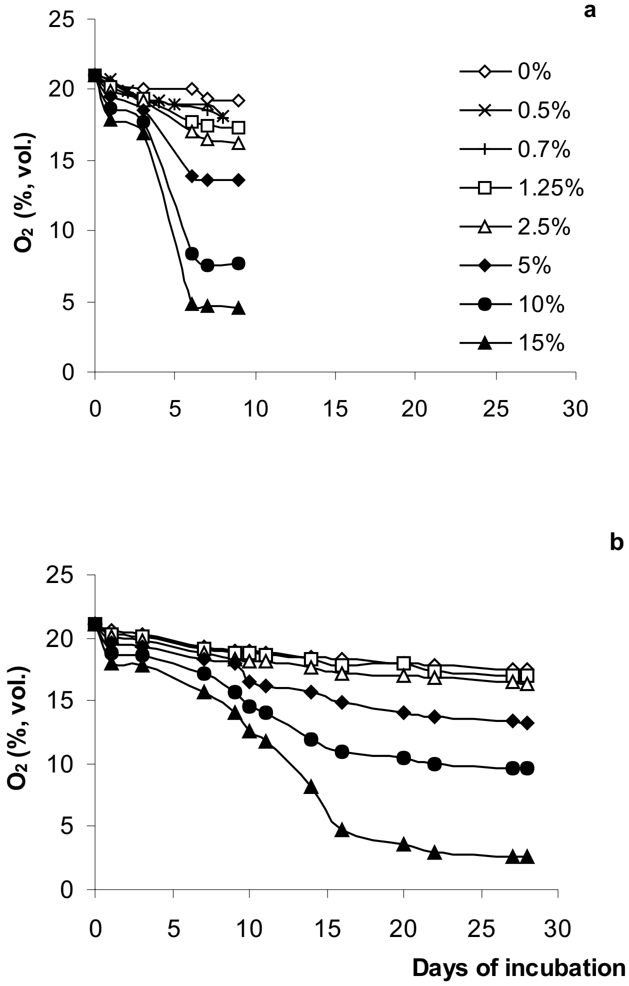

Fig. 4. The course of oxygen consumption (%, vol.) in the head-space during the incubation of the Mollic Gleysol (a) and the Haplic Podzol (b).

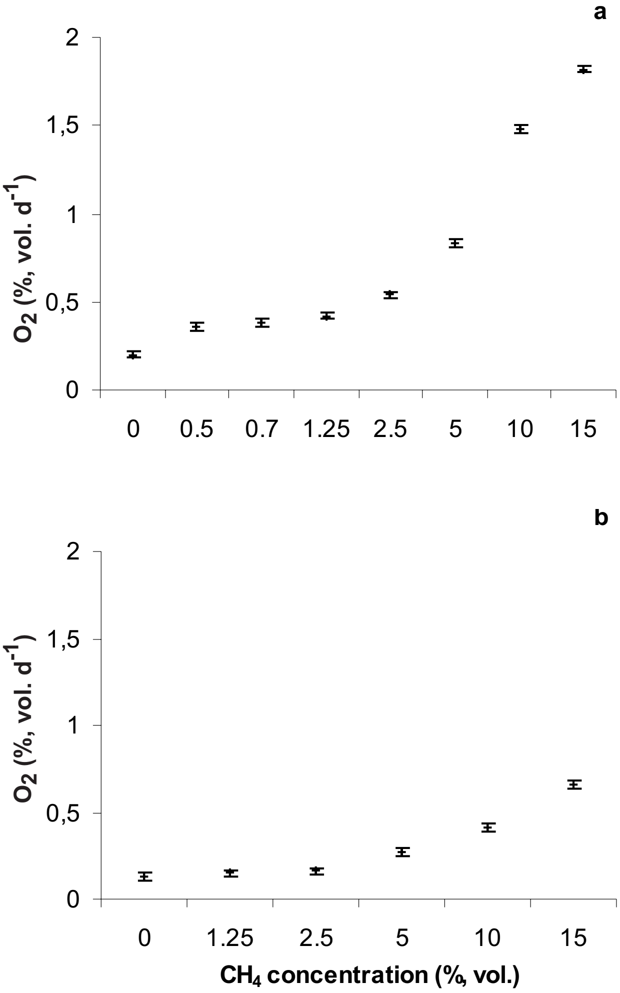

Fig. 5. Daily oxygen consumption (%, vol. d^{-1}) in the Mollic Gleysol (a) and the Haplic Podzol (b) as a function of CH_4 concentration. The bars represent 95% LSD confidence intervals.

significantly higher (0.88% O_2 d^{-1}) than in Haplic Podzol (0.33% O_2 d^{-1}, Fig. 6), which can confirm the difference in microorganisms occurrence in the investigated soils with respect to the threshold of methane concentration.

Methane oxidation is accompanied by O_2 consumption and CO_2 production according to the following equation:

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

Table 2 shows the rate of CH_4 and O_2 consumption and CO_2 production depending on the CH_4 addition and the kind of soil, expressed by ml of investigated gases per kg and per day and their ratios. Daily CO_2 production increased with increasing methane amendment and was proportional to CH_4 oxidation and ranged from 11.4 to 87.7 and from 10.6 to 38.6 ml CO_2 kg^{-1} d^{-1} for the Mollic Gleysol and the Haplic Podzol, respectively. The average daily CO_2 production was about 2.5 fold higher in the Mollic Gleysol than in the Haplic Podzol. The data showed different courses of the CH_4:O_2:CO_2 ratio in the two investigated soils. A slightly higher

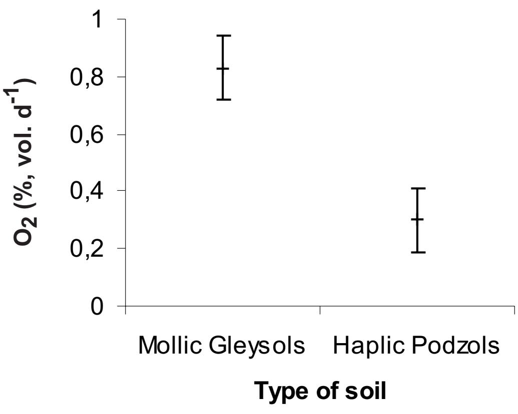

Fig. 6. Daily oxygen consumption (%, vol. d^{-1}) as a function of the type of soil. The bars represent 95% LSD confidence intervals.

T a b l e 2. The rate of CH_4 and O_2 consumption and CO_2 production depending on the CH_4 addition and type of soil

Soil	CH_4 amendment (%)	CH_4	O_2	CO_2	CH_4:O_2:CO_2*
		(ml kg^{-1} d^{-1})			
Mollic Gleysol	0	0	18.8	11.4	-
	1.25	23.0	39.1	20.4	1 : 0.88 : 0.39
	2.5	45.2	50.4	29.5	1 : 0.70 : 0.40
	5	84.7	78.0	46.2	1 : 0.70 : 0.41
	10	163.2	139.2	78.1	1 : 0.74 : 0.41
	15	124.8	171.5	87.7	1 : 1.22 : 0.61
Haplic Podzol	0	0	12.2	10.6	-
	1.25	1.6	13.9	11.5	1 : 1.06 : 0.58
	2.5	2.9	15.7	12.0	1 : 1.21 : 0.50
	5	10.3	26.2	18.8	1 : 1.36 : 0.79
	10	19.1	38.7	25.4	1 : 1.39 : 0.78
	15	39.0	62.4	38.6	1 : 1.29 : 0.72

* CH_4 : O_2 : CO_2 ratio was calculated for the O_2 consumption and CO_2 production as a difference between CH_4-amendment soil minus the control without CH_4 amendment.

O_2 utilization and CO_2 production per 1 ml of CH_4 oxidised was found in the Haplic Podzol, which was classified as a soil with low affinity to CH_4 oxidation. The soil (Mollic Gleysol) with the higher affinity to CH_4 oxidation was characterized by lower O_2 consumption and lower CO_2 production, except for the soil with 15% methane addition, where the investigated ratio was apparently higher, probably for the reason of the effect of inhibition by the surplus of the substrate. A comparable range of the CH_4: O_2:CO_2 ratio was found in the other publications (Stępniewski and Pawłowska, 1996; Brzezińska *et al*., 2004).

CONCLUSIONS

1. Higher methanotrophy and respiration activity was shown by Mollic Gleysol than by Haplic Podzol.

2. Methanotrophic activity increased with increasing CH_4 addition in the range of 0.5 to 10% initial methane for Mollic Gleysol and 1.25 to 15% in the case of Haplic Podzol.

3. The soils investigated were characterized by different requirements with respect to threshold methane concentration expressed as high affinity oxidation (Mollic Gleysol) and low affinity oxidation (Haplic Podzol).

4. Daily oxygen consumption increased with increasing methane concentration and was proportional to CH_4 oxidation.

5. The differences in methanotrophic activity mainly resulted from differences in organic matter content and methanotrophs existed in the investigated soils.

6. Daily CO_2 production increased with increasing methane amendment and was about 2.5 fold higher in the Mollic Gleysol than in the Haplic Podzol.

7. The investigated soils showed different courses of the CH_4:O_2:CO_2 ratio which was probably related to their affinity to CH_4 oxidation.

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