Kinetics of methane oxidation in selected mineral soils

A. Walkiewicz, P. Bulak, M. Brzezińska*, T. Włodarczyk, and C. Polakowski

Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland

A b s t r a c t. The kinetic parameters of methane oxidation in three mineral soils were measured under laboratory conditions. Incubations were preceded by a 24-day preincubation with 10% vol. of methane. All soils showed potential to the consumption of added methane. None of the soils, however, consumed atmospheric CH_4. Methane oxidation followed the Michaelis-Menten kinetics, with relatively low values of parameters for Eutric Cambisol, while high values for Haplic Podzol, and especially for Mollic Gleysol which showed the highest methanotrophic activity and much lower affinity to methane. The high values of parameters for methane oxidation are typical for organic soils and mineral soils from landfill cover. The possibility of the involvement of nitrifying microorganisms, which inhabit the ammonia-fertilized agricultural soils should be verified.

K e y w o r d s: soil, methane oxidation, kinetic parameters, methanotrophic activity

INTRODUCTION

Methane (CH_4) is the most abundant hydrocarbon in the atmosphere, and it is an important greenhouse gas contributing to an estimated 18-20% of postindustrial global warming (Knittel and Boetius, 2009). The atmospheric CH_4 concentration has risen from the background level to 1 782 ppb in 2006 (IPCC, 2007). Soil is both emitter and consumer of greenhouse gases (Włodarczyk *et al.*, 2011). The surface CH_4 emissions is estimated to reach 643 Tg year^{-1}, while microbial CH_4 oxidation in well-drained upland soils accounts for about 6% of the global atmospheric methane sink (Fowler *et al.*, 2009; IPCC, 2007).

Methane oxidizing bacteria (methanotrophs) play a significant role in the carbon cycling; they represent one of the largest biological sinks for methane in soils, and the only biological sink under aerobic conditions (Conrad, 2007). Methanotrophs are present in the aerobic soil layers, rhizo-

sphere, roots and stem bases of dryland and flooded rice agroecosystems (Vishwakarma and Dubey, 2010). Numerous molecular methods have been developed to study the diversity, structure and abundance of methanotrophic communities *in situ* (Steenbergh *et al.*, 2010). The key enzyme on methanotrophy is methane monooxygenase (MMO), which converts methane to methanol. The exact catalytic mechanism of methane oxidation is still hypothetical (Baani and Liesack, 2008). Methane monooxygenase, especially soluble sMMO, is remarkable in its broad substrate specificity, and catalyses oxidation of other alkanes, alkenes, alicyclic hydrocarbons, halogenated aliphatics, mono- and di-aromatics and others. This unique capability, *ie* catalyzing reactions of environmental importance, has attracted great attention for applied microbiologists and biochemical engineers, and a potential value of methanotrophs in environmental bioengineering for biodegradation of toxic compounds is studied (Jiang *et al.*, 2010).

Knowledge about the kinetics of an enzyme reaction with its substrate is centrally important to biochemists. Information about kinetics of enzyme reaction is commonly used to interpret the role of particular enzyme in metabolic control of the biological process (Psillos and Niedderer, 2003). The kinetics of many enzyme-catalyzed reactions are described by Michaelis-Menten equation with the maximum reaction rate defined as V_{max}, and the Michaelis constant, K_m, defined as the substrate concentration at which the reaction achieves half the maximal rate ($V_{max}/2$); K_m is independent on enzyme concentration, while V_{max} increases with the enzyme concentration increase. Traditionally, K_m is expressed in molarity, while several authors use such units as ppm and % vol. (Pawłowska and Stępniewski, 2006). Kinetic constants are derived experimentally. An important point can be made about the physiological implications of

---

*Corresponding author's e-mail: m.brzezinska@ipan.lublin.pl

enzyme kinetic constants. If the substrate concentration is known, then it is possible to predict from the K_m value whether or not an enzyme is saturated. This, in turn, would indicate whether the enzyme was likely the rate-limiting step in a given pathway. Also, a knowledge of the values of V_{max} for the various enzymes in a pathway would allow predictions to be made of the maximum obtainable flux of metabolites through a particular route (Smith and Wood, 1991).

Methane oxidation has been shown to follow Michaelis-Menten kinetics (Baani and Liesack, 2008; Saari *et al.*, 2004; Steenbergh *et al.*, 2010). Most known (cultured) methanotrophs have a low affinity for CH_4, and can not maintain their growth on CH_4 at atmospheric (ambient) concentration, but need the higher CH_4 concentrations. Although there are no pure cultures of high affinity CH_4 oxidizers, these organisms are known to exist in upland soils where they account for about 10% of the annual global CH_4 sink. The observed capacity for both types of oxidation kinetics in saturated soils and sediments has been assumed to be due to the presence of mixed methanotrophic populations (Megonigal *et al.*, 2004).

Because N fertilization, especially ammonium fertilizers, frequently reduces soil CH_4 consumption, agricultural soils have been regarded to be rather poor sink for methane. However, Chan and Parkin (2001) have shown that although the prairie and forest soils had the greatest potential to oxidize atmospheric concentrations of CH_4, but soils from many of the agricultural sites exhibited greater CH_4 oxidation activity when exposed to elevated CH_4 concentrations.

The aim of the paper was to study the kinetic parameters of methane oxidation in soils enriched with methane under controlled laboratory conditions.

MATERIALS AND METHODS

Three soils Mollic Gleysol, Haplic Podzol and Eutric Cambisol were collected in 2011 from a depth of 0-20 cm, air-dried and sieved to <2 mm. Soils were selected as characterized by a similar texture, which determines the in situ soil air-water conditions and, thus, strongly regulates soil methanotrophic activity. Basic soil properties are presented in Table 1.

The 3-g samples of air-dry soils were weighted into 20 cm^3 glass vessels and moistened with distilled water to obtain the field water capacity (FWC). Since aerobic methanotrophic bacteria require both oxygen and water, soils were incubated at field water capacity that optimize both air and moisture. In the conditions of Poland, FWC represents the water content which is bound in the soil at potential of 16 kJ m^{-3} (pF 2.2) (Walczak *et al.*, 2002). All the vessels were tightly closed with rubber stoppers and aluminium caps, and after adequate volumes of air from the vessels were removed to avoid overpressure, soils were enriched with methane. The samples were incubated at a constant temperature 25°C in the dark. The incubation was preceded by a 24-day preincubation of all samples under the same conditions with 10% methane in the headspace. When added CH_4 was consumed, all vessels were opened, and allowed to ventilate for 2 h. The bottles were then sealed and the procedure of soil enrichment was repeated; methane was added to obtain 0.5, 1.0, 1.5, 3.0, 5.0, and 10% (vol.), which corresponded to CH_4 range from 1.34 to 25.67 µmol CH_4 kg^{-1} d.m. soil. Additionally, samples without CH_4 additions were prepared to study the possibility of soils to oxidize atmospheric CH_4 (1.8 ppm).

Determining the instantaneous reaction rate is not feasible in practice and the rate is determined instead based on the rate of change of CH_4 concentration over time (Gulledge *et al.*, 2004). The initial methane oxidation rate was determined using the methane sampling times from 0 to 26 h (Steenbergh *et al.*, 2010), and the rates were obtained by dividing the CH_4 oxidation with incubation time (Xu and Inubushi, 2009). Lineweaver-Burk equation was used to determine the kinetic parameters of methane oxidation:

$$\frac{1}{v} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}},$$

where: K_m – the Michaelis constant, and V_{max} – the maximum reaction rate, v – CH_4 oxidation rate at a given substrate concentration [S]. Based on the values of the slope and intercept (K_m/V_{max} and $1/V_{max}$, respectively) obtained for each soil by regression of $1/v$ vs. $1/S$, the apparent K_m and V_{max} were calculated. The K_m and V_{max} were expressed in µmol CH_4 and µmol CH_4 g^{-1} h^{-1}, respectively.

Concentrations of CH_4 in the headspace was measured with gas chromatographs Shimadzu GC-14B (Japan) equipped with a flame ionization detector (FID) and GC-14A equipped with a thermal conductivity detector (TCD). Low, atmospheric CH_4 concentrations were detected by the FID; the gas components were separated on a column packed with a Porapak Q maintained at 80°C, and the temperature of the

T a b l e 1. Basic properties of tested soils

Soil types	Grain size distribution (%, dia in µm)			C_{org} (%)	pH (KCl)
	2 000-50	50-2	< 2		
Mollic Gleysol	74.8	21.7	3.44	3.93	7.71
Haplic Podzol	74.6	22.3	3.04	0.43	6.50
Eutric Cambisol	71.6	25.1	3.23	1.18	6.38

injector was 150°C. For higher CH_4 concentrations, TCD detector with a 2-m column (3.2 mm in dia) packed with Porapak Q was used with He as a carrier gas flowing at a rate of 40 $cm^3 min^{-1}$; the temperature of the column and detector were 40°C and 60°C, respectively (Brzezińska et al., 2011; 2012). The detector responses were calibrated using certified gas standards (Air Products) containing 10 ppm or 4% CH_4 in He.

Particle size distribution (FAO Classification) was measured using the Mastersizer 2000 (Malvern, UK) laser diffractometer within the size range of 0.02 μm to 2 mm (Bieganowski et al., 2010). The Hydro G dispersion unit was used with 1750 r.p.m. of the pump and 700 r.p.m. of stirrer (Sochan et al., 2012). Total organic carbon (C_{org}) was determined by TOC-VCPH analyzer (Shimadzu, Japan), and soil pH was measured in 1 mol KCl (1:2.5 mass) after 24-h stabilization at room temperature. All measurements were done in triplicate.

RESULTS AND DISCUSSION

Tested soils differed in their ability to the oxidation of added methane. During the preincubations, the 1- to 4-day lag periods before the onset of CH_4 oxidation were observed (Fig. 1). The lag phase typically occurs as a result of adaptation of soil microorganisms to added substrate (Steenbergh et al., 2010). The use of the preincubations allowed to reduce this period during following incubations with different CH_4 concentrations (Fig. 2). Among the tested soils, the Mollic Gleysol showed the highest methanotrophic activity. All added CH_4 was taken up during seven days of the incubation (Fig. 2a). Lower CH_4 enrichments (<1.5% vol.) were depleted already after two days, and nearly linear decline of the highest methane of 10% vol. was observed. The Haplic Podzol soil showed some slower decline of CH_4 over time, especially at elevated initial CH_4 in the headspace (5-10% vol.) (Fig. 2b). In turn, the Eutric Cambisol incubated with 10% CH_4 showed the slowest CH_4 consumption among tested soils, and needed 18 days to deplete high CH_4 amendments (Fig. 2c). The Haplic Podzol and Eutric Cambisol soils, contrary to the Mollic Gleysol, did not utilized all methane added at the lowest 0.5% vol. treatment, and there was still 0.2-0.3% vol. CH_4 in the headspace at the end of the incubation. Moreover, none of tested soils showed ability to consume the atmospheric methane.

The CH_4 oxidation rates were expressed in substrate saturation curves as a function of initial methane concentrations (Fig. 3a-c) and showed typical Michaelis-Menten characteristics. Lineweaver-Burk plots based on regression of $1/v$ vs. $1/S$ are presented as insets in Fig. 3. The apparent K_m and V_{max} values obtained for tested soils, as calculated from the slopes and intercepts of these linear equations are summarized in Table 2.
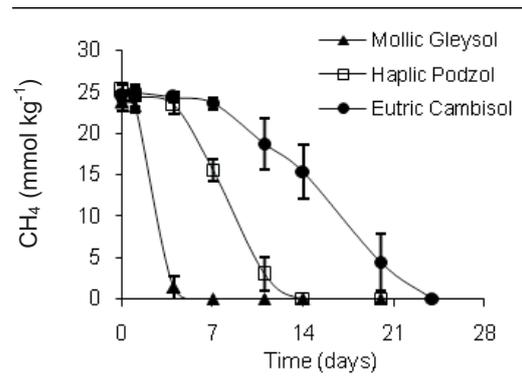

Fig. 1. Changes of CH_4 during the preincubation of tested soils with 10% (vol.) CH_4. Points are average values from three replications ± SD.
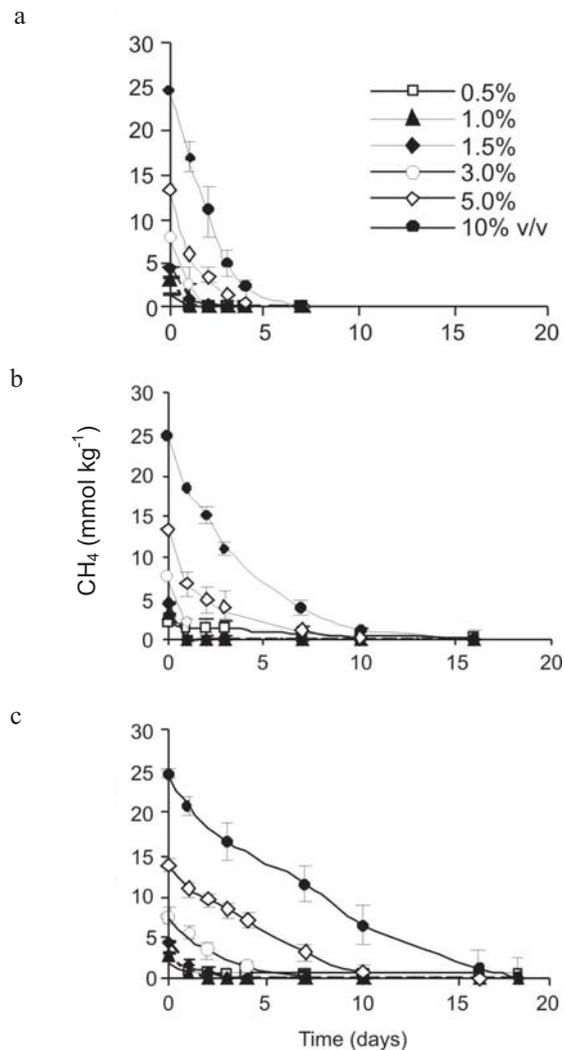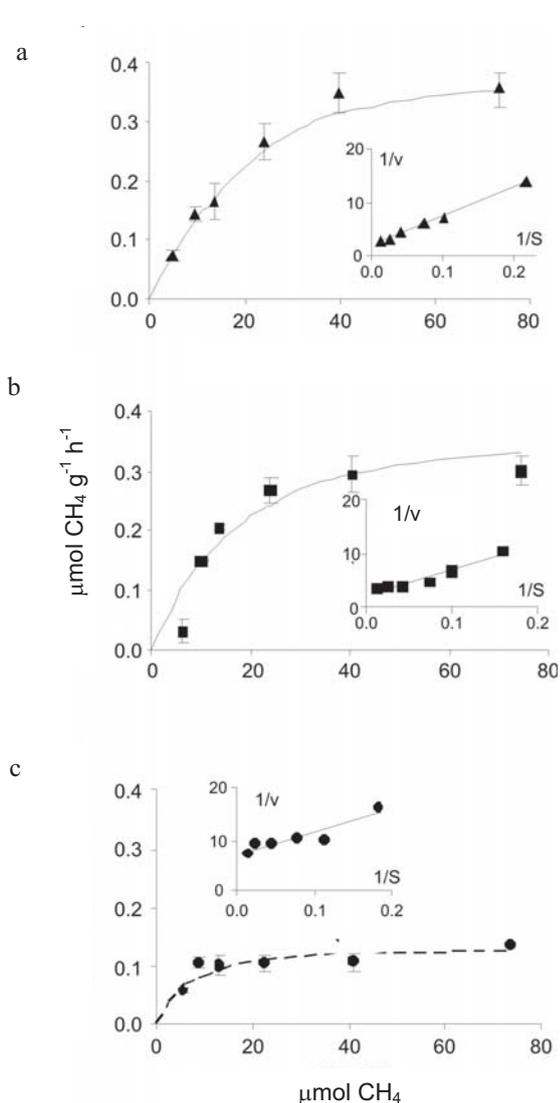

Fig. 2. Dynamics of CH_4 over the incubation of the: a – Mollic Gleysol, b – Haplic Podzol, c – Eutric Cambisol enriched with 0.5, 1.0, 1.5, 3.0, 5.0, and 10% (vol.) methane. Bars indicate standard deviations of the mean (n=3).

Fig. 3. Michaelis-Menten saturation curves of CH_4 oxidation in: a – Mollic Gleysol, b – Haplic Podzol, c – Eutric Cambisol. Each point represent average value from three replications ± SD. Insets: Lineweaver-Burk plots with linear functions to determine K_m and V_{max}: (a) y=55.5x+1.83, R^2=0.99, p<0.001; (b) y=49.0x+2.08, R^2=0.95, p<0.001; and (c) y=44.0x+7.22, R^2=0.83 p<0.05.

Table 2. Kinetic parameters of CH_4 oxidation in tested soils (K_m and V_{max}) calculated from linear regression of Lineweaver-Burk plots

Soil type	K_m (µmol)	V_{max} (µmol g^{-1} h^{-1})
Mollic Gleysol	30.66	0.550
Haplic Podzol	19.79	0.443
Eutric Cambisol	5.98	0.137

Cultured methanotrophs, classified into different types I and II depending on the intracellular membrane arrangement, pathways of carbon assimilation, and phospholipid fatty acid composition, typically inhabit the aerobic interfaces of methanogenic environments, such as natural wetlands and rice paddies. Because of the usually high methane supply in these environments, methane is oxidized with low affinity and high K_m values (>1 µmol CH_4). These K_m values are similar to those determined for cultured methanotrophs (K_m = 2-12 µmol CH_4) (Baani and Liesack, 2008). In turn, in upland soils, methanotrophic bacteria take up CH_4 directly from the atmosphere. Methane consumption in these soils is a small but a significant part of the global methane budget, comparable in magnitude to the estimated excess of emissions over sinks in recent years (29 Tg year^{-1}). In contrast to those methanotrophs in the aerobic interfaces of methanogenic environments, the methanotrophs active in dry, well aerated upland soils, for example, forest soils, consume methane with high apparent affinity and low K_m values of 0.01-0.28 µmol CH_4 (Baani and Liesack, 2008; Megonigal *et al.*, 2004). Recently, a type II methanotrophs able to adapt to K_m values close to those measured in upland forest soil was isolated from a humisol suggesting that the affinity of methanotrophic bacteria for CH_4 can vary according to the environmental conditions (Saari *et al.*, 2004). It also has been suggested that low- and high-affinity oxidations may be carried out by the same methanotrophs, in their case a type II methanotroph, but that affinity for CH_4 changes as a function of growth conditions. Moreover, it is well documented, that ammonia-oxidizing bacteria can also oxidize CH_4 as an alternative substrate for ammonia monooxygenase, perhaps making this group of microorganisms important in cycling of CH_4 in agricultural systems (Chan and Parkin, 2001).

All soils tested in our study showed the potential to methane oxidation. Relatively short lag phase of 1 to 4 days observed during preincubations indicated that methane oxidizers inhabiting these soils exhibit quite fast adaptation to high methane in the headspace. The Mollic Gleysol characterized by a high pH 7.71 showed the highest methanotrophic activity, while Eutric Cambisol characterized by pH 6.38 showed the lowest activity (Figs 1, 2). Such a difference in soil pH, however, probably can not be responsible for the difference in the CH_4 oxidation activity. Until recently, all cultured methanotrophs required a pH >5 and most were neutrophilic with optimum pH of 6.8-7.0. Yet, CH_4 oxidation at pH <5 has been demonstrated in acidic *Sphagnum*-bogs, and acidophilic methanotrophic bacteria have been isolated with an optimum activity between pH 4.5 and 5.5 (Megonigal *et al.*, 2004). Saari *et al.* (2004) observed for several mineral and organic soils that the pH optimum for methanotrophic activity varied from 3.9 to 7.5. Nevertheless, based on the study of 12 different soils, Chan and Parkin (2001) reported that in soils with pH less than 5.0, CH_4 oxidation activity was nearly eliminated.

Methane oxidation in tested soils followed Michaelis-Menten kinetics. The Eutric Cambisol reached highest for this soil activity at relatively low CH_4 concentrations, and no further increase of the activity was observed (Fig. 3). The apparent K_m value for this soil was 5.98 µmol, and V_{max} was 0.137 µMol g^{-1} h^{-1}. These values are comparable with kinetic parameters measured in other mineral soils (Gulledge *et al*., 2004; Megonigal *et al*., 2004; and papers therein). In turn, Haplic Podzol and, especially, the Mollic Gleysol showed CH_4 oxidation kinetics similar to those observed in organic soils (Bender and Conrad, 1992; Gulledge *et al*., 2004; Saari *et al*., 2004), as well as in some soils with agricultural origin and soils from landfill cover (Pawłowska and Stępniewski, 2006; and references therein). CH_4 oxidation in these two soils may be described as low affinity activity with high K_m (19.8-30.7 µmol) and high V_{max} values (0.44-0.55 µmol g^{-1} h^{-1}). Similar activities with high K_m values up to 2.9% vol. in gaseous phase have been observed in a coarse sand purged with 99% methane (Pawłowska and Stępniewski, 2006). For comparison, if express our results in the same unit, then the constants obtained in our experiment gave comparable values of K_m =2-3% vol. Very high K_m values even up to 66 µmol CH_4 were observed for forested peat soil and agricultural humisol (Gulledge *et al*., 2004; Saari *et al*., 2004).

As Bradford *et al*. (2001) pointed, the kinetic parameters for soil CH_4 oxidation provide valuable information regarding the dynamics of CH_4 oxidation and production within a soil profile. By quantifying the kinetics, it could be determined whether a soil was exposed only to atmospheric and sub-atmospheric concentrations of CH_4, with high affinity methanotrophic activity then dominating. The additional presence of low affinity activity would imply that a significant CH_4 source was present within the soil, possibly due to biological CH_4 production, as has been observed for many peat systems (Bradford *et al*., 2001). The Mollic Gleysol tested in our study was characterized by a high C_{org} content of 3.93%. Typically, Mollic Gleysols are fertile soils rich in organic matter, and soils of high microbial biomass C and N contents show, as a rule, high biological quality (Gajda and Przewłoka, 2012). These properties may promote creation of anaerobic microsites in deeper soil layers, when soil get wet. As methanogenic archaea are present also in agricultural soils, and their growth is stimulated in periods of higher soils moisture content, they may be a source for CH_4 diffusing through the soil profile. A surface layer, in turn, may be inhabited by populations of aerobic methanotrophic bacteria, adapted to CH_4 concentrations higher than atmospheric. However, low affinity kinetics observed in the Haplic Podzol can not be explained by high fertility, as this soil showed only 0.43% C_{org}. In fact, tested soils did not oxidize methane at ambient concentration, and did not express the high affinity activity which is typical for upland soils exposed to only atmospheric CH_4 mixing ratios. However, Chan and Parkin (2001) showed that under an elevated CH_4 atmosphere, N may actually stimulate CH_4 oxidation activity and suggest that the CH_4 oxidation activity carried out under elevated atmospheric CH_4 levels may be predominantly due to nitrifying bacteria, especially in the agricultural systems. Regular ammonia-N fertilizer application to agricultural soils nay result in increased populations of nitrifying bacteria, which will coincidently metabolize CH_4, and may also stimulate methanotrophic bacterial populations, as both methanotrophs and nitrifying bacteria can oxidize both CH_4 and NH_4^+ (Chan and Parkin, 2001). Therefore, additional studies are necessary to better understanding the processes of methane oxidation in agricultural soils.

CONCLUSIONS

1. Tested soils, Mollic Gleysol, Haplic Podzol and Eutric Cambisol showed potential to oxidize added methane up to 10% vol. in the headspace.

2. None of the soils consumed atmospheric (ambient) methane.

3. Methane oxidation followed Michaelis-Menten kinetics.

4. The Eutric Cambisol showed higher than other soils affinity to methane, characterized by relatively low K_m and V_{max} values of 5.98 µmol and 0.137 µmol g^{-1} h^{-1}, respectively.

5. The Mollic Gleysol oxidized added methane most rapidly with a low affinity activity characterized by a high apparent K_m=30.7 µmol and V_{max}=0.550 µmol g^{-1} h^{-1}.

REFERENCES

Baani M. and Liesack W., 2008. Two isozymes of particulate methane monooxygenase with different methane oxidation kinetics are found in *Methylocystis* sp. strain SC2. PNAS, 105, 10203-10208.

Bender M. and Conrad R., 1992. Kinetics of CH_4 oxidation in oxic soils exposed to ambient air or high CH_4 mixing ratios. FEMS Microbiol. Ecol., 101, 261-270.

Bieganowski A., Ryżak M., and Witkowska-Walczak B., 2010. Determination of soil aggregate disintegration dynamics using laser diffraction. Clay Minerals, 45, 23-34.

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., Nosalewicz M., Pasztelan M., and Włodarczyk T., 2012. Methane production and consumption in loess soil at different slope position. Sci. World J., ID 620270.

Brzezińska M., Rafalski P., Włodarczyk T., Szarlip P., and Brzeziński K., 2011. How much oxygen is needed for acetylene to be consumed in soil? J. Soils Sediments, 11, 1142-1154.

Chan A.S.K. and Parkin T.B., 2001. Methane oxidation and production activity in soils from natural and agricultural ecosystems. J. Environ. Qual., 30, 1896-1903.

Conrad R., 2007. Microbial ecology of methanogens and methanotrophs. Adv. Agron., 96, 1-63.

Fowler D., Pilegaard K., Sutton M.A., 2009. Atmospheric composition change: ecosystems-atmosphere interactions. Atmospheric Environ., 43, 5193-5267.

Gajda A.M. and Przewłoka B., 2012. Soil biological activity as affected by tillage intensity. Int. Agrophys., 26, 15-23.

Gulledge J., Hrywna Y., Cavanaugh C., and Steudle P.A., 2004. Effects of long-term nitrogen fertilization on the uptake kinetics of atmospheric methane in temperate forest soils. FEMS Microbiol. Ecol., 49, 389-400.

IPCC, **2007.** Intergovernmental Panel on Climate Change, Climate change 2007: the scientific basis, Contribution of Working Group 1 to the 4th Assessment Report, Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, UK.

Jiang H., Chen Y., Jiang P., Zhang C., Smith T.J., Murrel J.C., and Xing X-H., 2010. Methanotrophs: Multifunctional bacteria with promising application in environmental bioengineering. Biochem. Eng. J., 49, 277-288.

Knittel K. and Boetius A., 2009. Anaerobic oxidation of methane: progress with an unknown process. Ann. Rev. Microbiol., 63, 311-334.

Megonigal J.P., Hines M.E., and Visscher P.T., 2004. Anaerobic metabolism: linkages to trace gases and aerobic processes. In: Biogeochemistry (Ed. W.H. Schlesinger). Elsevier-Pergamon Press, Oxford, UK.

Pawłowska M. and Stępniewski W., 2006. An influence of methane concentration on the methanotrophic activity of a model landfill cover. Ecol. Eng., 26, 392-395.

Psillos D. and Niedderer H., 2003. Teaching and Learning in the Science Laboratory. Kluwer Academic Press, New York, USA.

Saari A., Rinnan R., and Martikainen P.J., 2004. Methane oxidation in boreal forest soils: kinetics and sensitivity. Soil Biol. Biochem., 36, 1037-1046.

Sochan A., Bieganowski A., Ryżak M., Dobrowolski R., and Bartmiński P., 2012. Comparison of soil texture determined by two dispersion units of Mastersizer 2000. Int. Agrophys., 26, 99-102.

Smith C.A. and Wood E., 1991. Molecular and Cell Biochemistry. Biological Molecules. Chapman and Hall Press, London, UK.

Steenbergh A.K., Meima M.M., Kamst M., and Bodelier L.E.P., 2010. Biphasic kinetics of a methanotrophic community is a combination of growth and increased activity per cell. FEMS Microbiol. Ecol., 71, 12-22.

Vishwakarma P. and Dubey S.K., 2010. Diversity of methanotrophs in urea fertilized tropical rice agroecosystem. Indian J. Microbiol., 50, 205-211.

Walczak R., Ostrowski J., Witkowska-Walczak B., and Sławiński C., 2002. Spatial characteristic of hydrophysical properties in arable mineral soils in Poland as illustrated by field water capacity (FWC). Int. Agrophysics, 16, 151-159.

Włodarczyk T., Stępniewski W., Brzezińska M., and Majewska U., 2011. Various textured soil as nitrous oxide emitter and consumer. Int. Agrophys., 25, 287-297.

Xu X. and Inubushi K., 2009. Ethylene oxidation, atmospheric methane consumption, and ammonium oxidation in temperate volcanic forest soils. Biol. Fertil. Soils, 45, 265-271.