ISSN 0026-2617, Microbiology, 2010, Vol. 79, No. 6, pp. 822–829. © Pleiades Publishing, Ltd., 2010.
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EXPERIMENTAL ARTICLES

Dynamics of Microbial Oxidation of Methane in the Water of Stratified Lakes

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Received December 22, 2009

Abstract—Fluctuations of methane (CH_4) concentration and the dynamics of microbial methane oxidation (MO) were investigated in the water column of freshwater stratified lakes of different trophicity levels during various seasonal periods and throughout the diurnal cycle. Characteristics of vertical CH_4 distribution and ranges of methane transformation rates were determined and found to depend upon the lake productivity as well as seasonal and daily fluctuations of hydrological and hydrochemical parameters. The highest rate of MO was registered in highly eutrophic lakes during summer stagnation under conditions of formation of a distinct metalimnial water layer with MO up to 0.4–1.2 ml CH_4/(l day). Under the same conditions, a maximum amount of bacterioplankton (6–13 × 10^6 cells/ml) was detected and CO_2 bacterial dark assimilation (DA) reached 50–72 μg C/(l day). In the metalimnion layer, a strong correlation ($R = 0.74$) was revealed between diurnal fluctuation dynamics of MO and DA.

Keywords: lakes, methane distribution in water, methane oxidation dynamics.

DOI: 10.1134/S0026261710060159

Investigations of the methane cycle in freshwater systems, where methane is the major terminal product of anaerobic destruction of organic matter, revealed the ecological importance of CH_4 not only in silts, but in water as well [1]. Heterogeneity of methane distribution and its transformation processes in the water column of stratified lakes was demonstrated [2–4]. However, there is a significant lack of knowledge on the characteristic ecological parameters and scales of bacterial methane oxidation in water bodies of various types. There are occasional observations on seasonal fluctuations in the processes and almost no data on the diurnal cycles of methane distribution over the water depth.

The aim of the present work was to study the dynamics of methane distribution and its oxidation processes in the water column of stratified lakes of varying trophic states.

MATERIALS AND METHODS

The work was carried out in a group of dimictic lakes situated in Latvia (Dridzas and Vishki), Estonia (Linojarv), and the Upper Volga basin (Pleshcheevo and Vidogoshch) during various seasons from 1987 to 2000. Most of the observations were made during the summer period in deep-water parts of the lakes under maximum heating of the epilimnion and stable temperature stratification.

Water samples were collected from different horizons (with 0.5- to 2-m steps) with 2- or 0.7-l (for the metalimnion) Perspex Ruthner's bathometers. The concentration of dissolved methane was determined by the phase equilibrium method [5] on a Chrom-5 gas chromatography system equipped with a flame ionization detector. The gases were separated on 2.5-m columns filled with the Porapak-N adsorbent for light hydrocarbon analysis at 35°C in helium flow (30 ml/min).

The rate of bacterial methane oxidation (MO) was assayed by CH_4 decrease in experimental flasks incubated in situ in comparison with the control [6–8]. For this purpose, water from the bathometer was carefully (avoiding air bubbles) poured into three-times-rinsed 60-ml glass vials and then covered with silicone stoppers and locking caps, with excess water removed through injection needles. Then, the control samples were fixed with 0.1 ml of saturated $HgCl_2$ solution and the experimental samples (three repeats per sampling point) were incubated under ambient conditions for 4–48 h depending on the season and saprobity of the site under study. Some of the experiments were performed in an extended mode, adding a parallel series of samples containing an inhibitor of MO, allylthiourea (ATU), at 1 mg/ml [9]. This step was required to take into account methane formation under microaerobic conditions occurring both in the metalimnion and in other water layers [4, 10].

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General characteristics and trophic state parameters of lake water during the period of maximum summer stratification

Parameters	Lakes				
	Dzirdas	Vishki	Pleshcheevo	Vidogoshch	Linojarv
Flat-water area, km^2	7.4	3.6	51.4	0.21	0.03
Maximum depth, m	65	20	25	16	11
Water pH	7.8–8.4	7.9–8.8	7.9–9.2	7.1–8.6	6.2–9.5
CH_4 concentration, ml/l	0.0009–0.03	0.004–0.14	0.004–0.48	0.03–1.1	0.02–19.5
*Primary production, mg C/(l day)	0.03–0.11	0.08–0.12	0.23–0.75	0.39–1.14	0.63–2.45
*Organic matter decomposition, mg C/(l day)	0.05–0.08	0.09–0.14	0.11–0.34	0.53–0.95	0.43–1.65
TAB, 10^6 cells/ml	0.6–1.1	0.7–1.9	1.3–3.8	1.6–6.1	3.9–16.5
CO_2 dark assimilation, μg C/(l day)	0.6–1.1	0.9–6.4	1.1–6.3	4.8–72	6.6–68
Trophic state index	Oligomesotrophic	Mesotrophic	Mesoeutrophic	Eutrophic	Hypereutrophic

* In the epilimnion.

After incubation, the samples were fixed and the vials were stored upside down until laboratory analysis. Prior to analysis, a gaseous phase was created in the vials by removing 10 ml of water with a syringe and substituting it with an inert gas (argon or helium). The samples were shaken vigorously and incubated at room temperature for 2–3 h to equilibrate the gas partial pressure at the interphase. Methane consumption (or formation) was calculated according to known formulas [5, 7].

The rate of dark CO_2 assimilation (DA) by bacterioplankton was determined by the standard radiocarbon technique [7]. The experimental scheme was as follows. The water was dispensed into two 100-ml vials of dark glass with silicone stoppers and locking caps, incubated for 1 h in the dark, and then supplemented with 0.5 ml of the working solution of $Na_2^{14}CO_3$, 9.5 × 10^6 cpm/ml. Further incubation was performed in situ for 10–14 h in lightproof bags. At the end of the experiment, the samples were fixed with formaldehyde and filtered under low vacuum through no. 7 Synpor membranes (0.3-μm pore diameter). The membranes were washed with 0.1 N HCl on filter paper and dried, and the membrane activity was measured using a Mark-3 scintillation counter.

The daily DA per unit of water volume was calculated according to the formula

$$DA = \frac{rC_{carb}24}{Rt}, \mu g\ C/(l\ day),$$

where r stands for the membrane radioactivity after filtration of the whole sample, cpm; C_{carb} is the concentration of carbonate and bicarbonate, μg C/l; 24 is the coefficient to convert to diurnal value; R is the working isotope activity, cpm; and t is the exposure time, h.

The total amount of bacterioplankton (TAB) was determined by the direct erythrosine method on membrane filters with 0.2-μm pore size under an Ergaval microscope at 10 × 100 magnification. Primary production of organic matter by phytoplankton photosynthesis and its decomposition in the water were evaluated by the oxygen method [7].

Other physicochemical characteristics were obtained using a KL-115 oxygen meter with a thermistor, a Radelkis ionomer, and a Sanare BOD tester.

RESULTS AND DISCUSSION

The studied dimictic water bodies differed considerably in their morphometry and the physicochemical characteristics of the water, as well as in the rates of phytoplankton photosynthesis and bacterial decomposition of organic matter. Increasing trophicity of the lakes results in increased methane concentration, bacterial density, and general production characteristics together with higher variability of these parameters, which is particularly pronounced in the periods of summer stagnation evidencing a more complex structure of deep water masses during this period (table).

In the less productive Dridzas and Vishki lakes, even under conditions of temperature stratification, the vertical distribution of bacterioplankton and of the methane cycle quantitative characteristics was rather homogeneous along the water column. Only in the near-bottom layers, where oxygen concentration in the water decreased somewhat, did the CH_4 concentration and the rate of its oxidation by bacteria increase slightly (Figs. 1a and 1b), up to 20–50 μl/l and 3–10 μl/(l day), respectively. These values are similar to those of holomictic mesotrophic water bodies [8].

In the lakes of higher trophic state, where water stratification was registered throughout almost the entire summer period [3], metalimnial peaks in bacterioplankton amount and methane oxidation rate (Figs. 1c–1e) were observed above the methane dome (at the lower limit of the thermocline). Here, within a relatively narrow water layer (0.3–1 m), the MO rate

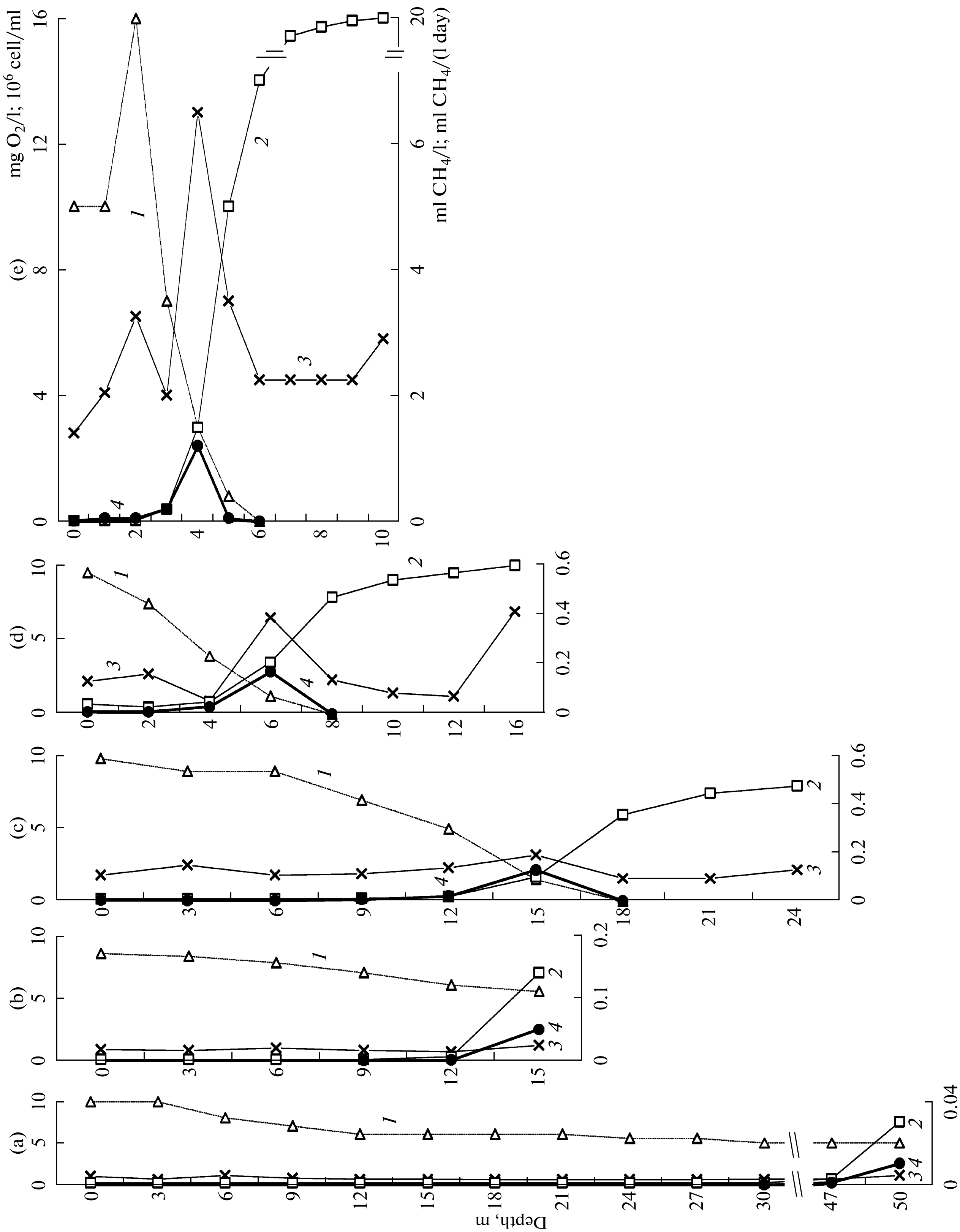

Fig. 1. Vertical distribution of O_2, mg/l (*1*); CH_4, ml/l (*2*); bacterioplankton, 10^6 cells/ml (*3*); and methane oxidation, ml CH_4/(l day) (*4*), in waters of lakes of various trophicity during the summer stratification period in the lake Dridzas (a), Vishki (b), Pleshcheevo (c), Vidogoshch (d), and Linojarv (e).

in certain periods of observation increased up to 0.2–1.2 ml CH_4/(l day), exceeding the values in adjacent horizons by more than an order of magnitude. These water bodies, namely, mesoeutrophic Lake Pleshcheevo, eutrophic Lake Vidogoshch, and hypereutrophic Lake Linojarv, were chosen as subjects for a more detailed study of dynamics of the microbial process in the CH_4 cycle.

In a number of expeditions, studies in Lake Pleshcheevo continued from May through September. In black silts of its kettle, methanogenesis was registered in all seasons and reached its maximum toward the end of the summer stagnation period when CH_4 production was 200 ml/(m^2 day) [11]. According to our observations, the further fate of the gas varied depending on the hydrological situation and oxygen supply regime during the sampling period.

In spring and early simmer, when the water was mixed over the entire column and the silts were well aerated, the generated methane was almost entirely consumed by the methanotrophic community of the silt surface [11]. The methane concentration and oxidation rate in the water were very low, not exceeding 3–7 μl CH_4/l and 0.1–0.3 μl CH_4/(l day), respectively, in the major part of the vertical profile. Close to the bottom, the gas concentration and MO value were slightly higher (Fig. 2a). With development of the temperature stratification and decrease in O_2 content in the near-bottom layers, progressive formation of the methane dome was observed with CH_4 concentration increasing up to 100–170 μl/l. While the zone of active methane oxidation in this period gradually increased, spreading by 10–12 m from the silts, the maximum MO was still registered immediately above the bottom in microaerobic water layers (Figs. 2b and 2c).

During the second half of summer, powerful destructive processes resulted in a sharp decrease in the amount of dissolved oxygen in the aphotic zone of Lake Pleshcheevo, leading to completed separation of the water depth into the aerobic epilimnion and the anaerobic hypolimnion. In the area of pronounced oxycline, a narrow microaerobic metalimnion was formed, where microbial MO processes reached the maximum rate of 50–150 μl CH_4/(l day) (Figs. 2d and 2e). In other words, a methanotrophic occlusive horizon [11–13] was observed, where most of the CH_4 coming from silts was subjected to oxidation and its concentration dropped by more than an order of magnitude. As a result, despite the considerable increase of methanogenesis in silts, the methane concentration in the epilimnion waters remained low, 5–10 μl CH_4/l, which is close to characteristic values for oligomesotrophic water bodies [14, 15]. During the autumn circulation period, the whole complex structure of the lake water masses was destroyed and the quantitative characteristics of the methane cycle at the time period reproduced the spring records (Fig. 2e).

The characteristic features of the diurnal cycle of CH_4 vertical distribution MO in the water column and during the summer period were studied in the hypereutrophic Lake Linojarv, where methane production in silts reached 3.4 l/(m^2 day) [11]. During the measurements carried out every 6 h, much attention was drawn to the 0- to 6-m zone, since oxygen did not reach below 4.5–5 m during this period of the year.

During the day insolation period, when the photosynthetic activity of the lake phytoplankton was high enough and its maximum was registered in the lower part of the epilimnion [3, 12], O_2 produced penetrated into deeper water layers and the progressively increasing metalimnion zone reached 4–4.5 m (Fig. 3) by 16 h. By this time, methane oxidation reached its maximum of up to 0.6–1.2 ml CH_4/(l day) in some metalimnion samples and the methane dome descended. Then, decreased insolation and fading phytoplankton activity resulted in the narrowing of the metalimnion zone and its elevation toward the upper water layers. The methane oxidation rate by the end of night period decreased 3–4-fold, and the methane dome rose up to the level of 2–2.5 m (Fig. 3), terminating the diurnal cycle of the vertical dynamics of methane oxidation.

Experiments in Lake Linojarv demonstrated that the location of the methanotrophic occlusive layer and the magnitude of the associated peak of CH_4 oxidation varied with the time of day and depended mostly upon the supply of dissolved oxygen to the metalimnion bacterial community. It is important to note that the most representative data on MO evaluation in the microaerobic zone were obtained in short-term experiments (4–6 h) when O_2 content in the vials remained above 0.5 mg/l. Data obtained at longer sample incubation times produced underestimated values when calculated per time unit.

The complex vertical dynamics of MO in waters of the lakes under study implies a different significance of these processes in the functioning of bacterioplankton communities of different zones of the water bodies. To explore the problem, an experiment was set up in eutrophic Lake Vidogoshch, where CH_4 production in silts in summer was 1.2 l/(m^2 day) [11]. The correlation between days-long dynamics of the processes of MO and CO_2 dark assimilation as a general characteristics of bacterioplankton functioning was studied [16]. The samples were collected every 6 h over 5 days in three horizons—namely, the surface (0.5 m), the midepilimnion (3 m), and the microaerobic metalimnion (5–6 m). The position of the latter was controlled by the oxygen meter probe (O_2 ~2–3 mg/l).

In the surface waters of the lake, where CH_4 concentration usually does not exceed 30 μl/l, the methane oxidation level remained extremely low and without significant fluctuations throughout the experiment (Fig. 4a). In the midepilimnion with methane concentration of 40–80 μl/l, the rate of MO increased insignificantly from time to time, although no rhythmics or causal factors were revealed. Neither were they found to be related to the dynamics of CO_2 dark assimilation

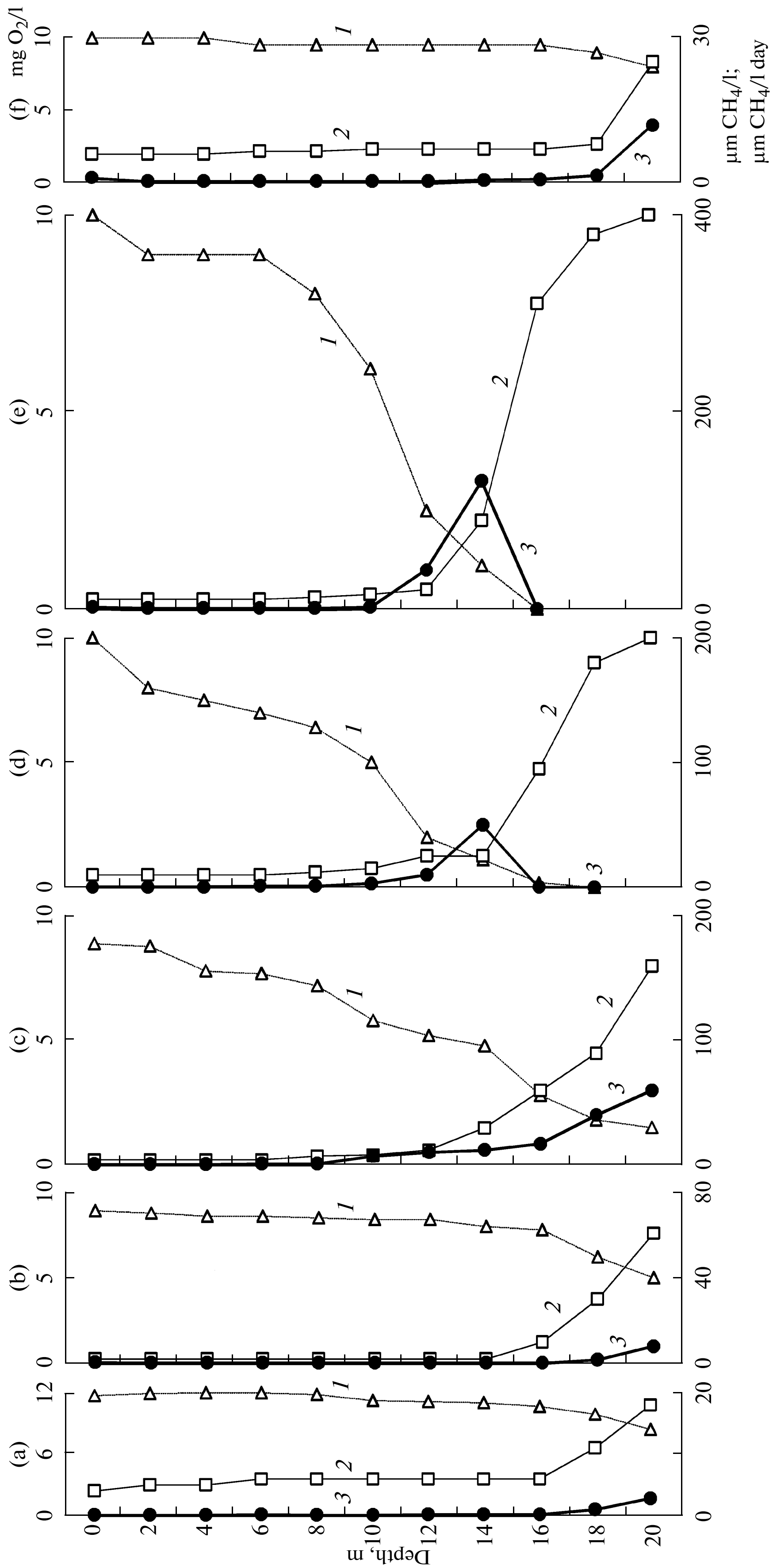

Fig. 2. Seasonal dynamics of vertical distribution of O_2, mg/l (*1*); CH_4, μl/l (*2*); and methane oxidation, μl CH_4/(l day) (*3*) in the water of Lake Pleshcheevo. Sampling dates are May 20–21 (a), June 23–24 (b), July 27–28 (c), August 5–6 (d), August 20–21 (e), and September 15–16 (f).

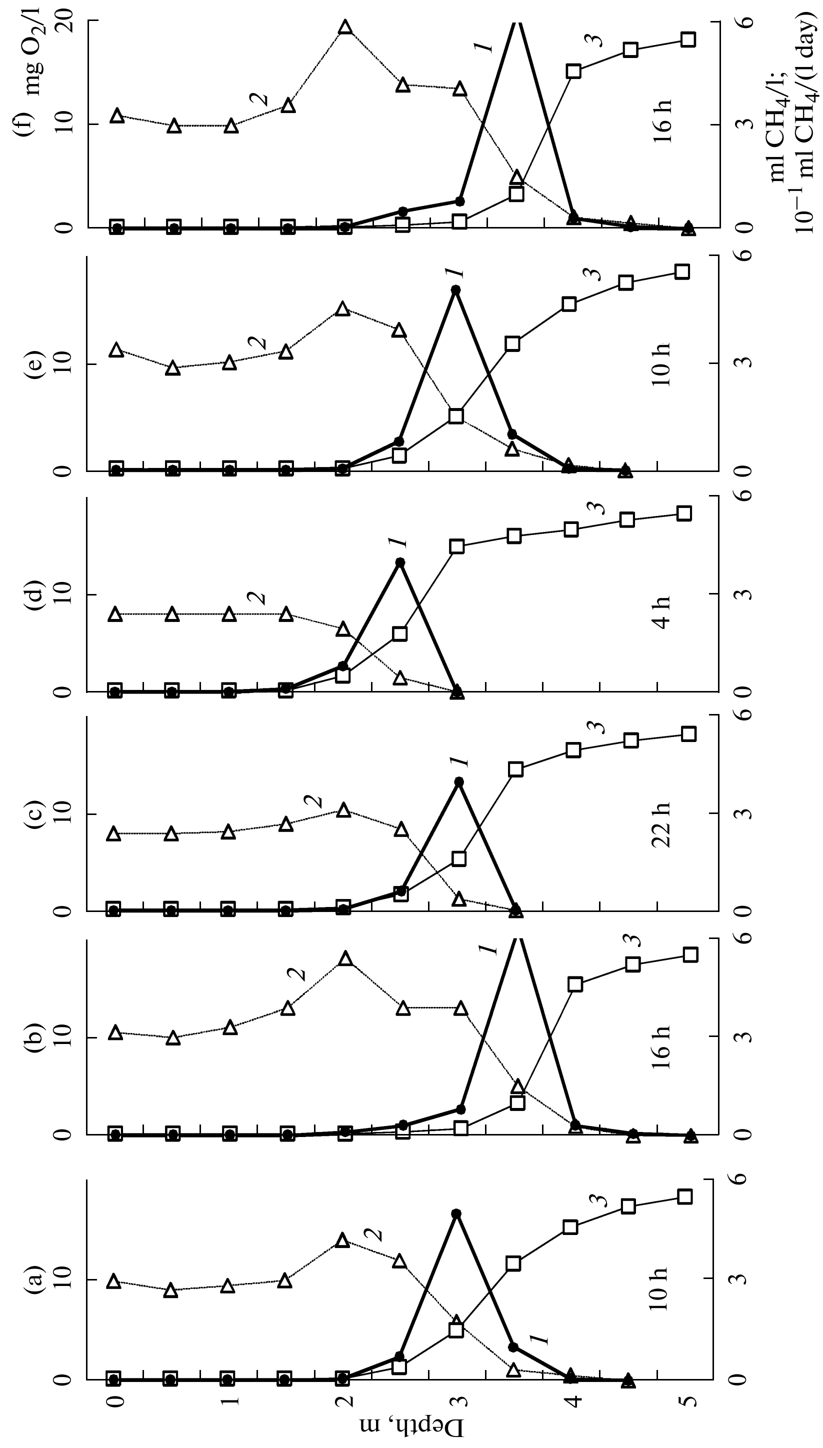

Fig. 3. Daily cycle of vertical dynamics of methane oxidation, ml CH_4/(l day) (*1*); dissolved O_2 concentration, mg/l (*2*); and CH_4 concentration, ml/l (*3*), in the water of Lake Linojarv (0-6 m deep layer). Time of the experiment start is indicated in brackets.

(Figs. 4a and 4b), which was generally high, reaching up to 3 μg C/(l h) in certain periods of observation. Evidently, in the presence of abundant products of photosynthesis, DA processes in these water layers proceed mainly at the expense of the heterotrophic bacterial community.

In the metalimnion, methane arriving from the profundal was oxidized rather rapidly with a distinct rhythm in the dynamics of the process. The minimal MO rate was detected in early morning hours at low phytoplankton activity, and the maximal in the afternoon when photosynthesis was the most intense and

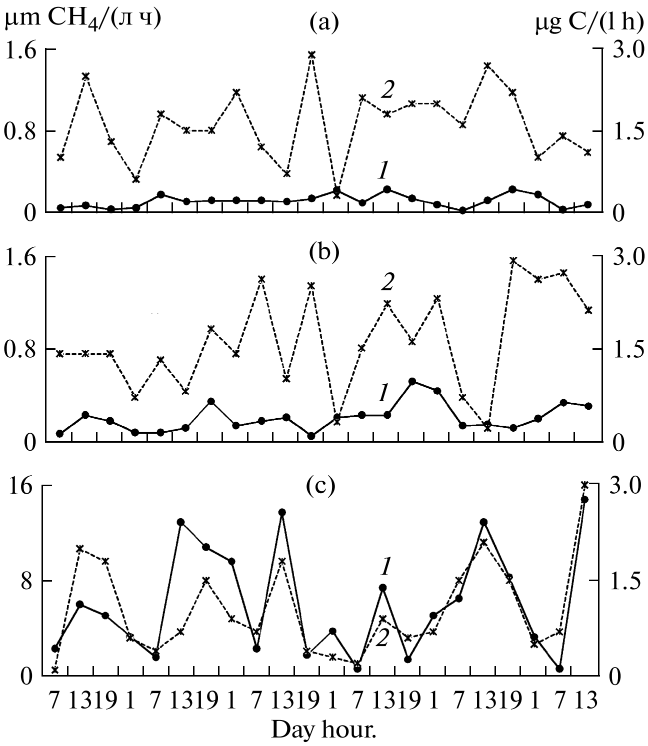

Fig. 4. Days-long dynamics of methane oxidation, μl CH_4/(l day) (*1*), and bacterial dark CO_2 assimilation, μg C/l h (*2*) at the surface (0-0.25 m) (a), in the epilimnion (3 m) (b) and in the metalimnion (5-6 m) (c) layers. Time of the experiment start is indicated along the *x* axis.

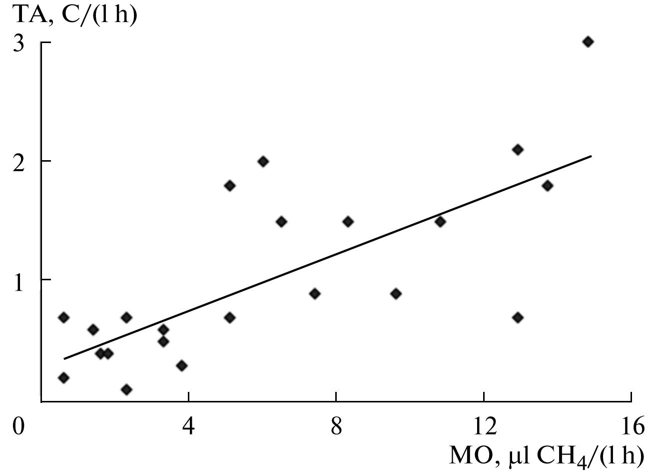

Fig. 5. Correlation between the rates of bacterial methane oxidation (MO) and dark CO_2 assimilation (DA) in the metalimnion waters of Lake Vidogoshch. Correlation coefficient R is 0.74.

the oxygen produced reached the methane dome. Similar rhythms were revealed in the dynamics of bacterial CO_2 assimilation (Fig. 4c). Analysis of the obtained data (Fig. 5) revealed high correlation between MO and DA processes in metalimnion ($R = 0.74$), evidencing the dominating role of the methane-oxidizing community in the functioning of bacterioplankton of this zone. Even the roughest calculations [3, 8] of the obtained data allow it to be assumed that 90–95% of the consumed oxygen is spent on methane oxidation.

Thus, our study of a number of dimictic lakes of different trophicity levels revealed that the profiles of methane distribution and methane oxidation rate in the water column depend on the water body productivity, oxygen supply regime, and CH_4 currents from the silts. In low-productivity water bodies, even during periods of stratification, the water mass is rather homogeneous in terms of the characteristics of the methane cycle. In highly trophic lakes, during summer stratification of waters, a narrow metalimnial zone is formed characterized by the maximum values of total bacterioplankton amount and CH_4 oxidation rate or a methanotrophic occlusive layer is formed where the gas concentration decreases sharply and TBA increases by from three to four times in comparison to the adjacent horizons. In waters of this layer, which changes its position during the day depending on the cycles of phytoplankton activity and oxygen supply, in the afternoon hours MO rates reach 0.4–1.2 ml CH_4/(l day) and the bacterioplankton amount reaches $6–17 \times 10^6$ cells/ml, which evidences its importance in the trophodynamics of the studied lakes. Consumption of dissolved O_2 in the process of methane oxidation reaches 90–95% from the total aerobic destruction [3], which in combination with the data on CO_2 dark assimilation suggests the dominating role of the methanotrophic community in the functioning of bacterioplankton of the metalimnion zone. In general, it becomes evident that the bacterial processes of methane oxidation have ecological significance both in productive lakes [1, 2] and in most other water bodies [3, 4, 8, 11, 15].

ACKNOWLEDGMENTS

The author is grateful to E.S. Bikbulatova, A.Yu. Gorbenko, and T.S. Maslennikova, Institute for Biology of Inland Waters, Russian Academy of Sciences, for their assistance in a number of experiments in Lake Vidogoshch.

The work was supported by the Russian Foundation for Basic Research, project no. 08-05-00079.

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