Limnol. Oceanogr., 51(2), 2006, 1195–1204

Methanotrophic activity in relation to methane efflux and total heterotrophic bacterial production in a stratified, humic, boreal lake

Paula Kankaala[1]
Lammi Biological Station, University of Helsinki, FIN-16900 Lammi, Finland

Jussi Huotari and Elina Peltomaa
Department of Ecological and Environmental Sciences, University of Helsinki, Niemenkatu 73, FIN-15140 Lahti, Finland

Tuomo Saloranta
Norwegian Institute for Water Research (NIVA), PB 173 Kjelsås, 0411 Oslo, Norway

Anne Ojala
Department of Ecological and Environmental Sciences, University of Helsinki, Niemenkatu 73, FIN-15140 Lahti, Finland

Abstract

We studied methanotrophic activity in the water column in relation to heterotrophic bacterial production and efflux of methane (CH_4) from the lake surface in a small, stratified, humic, boreal lake (Valkea-Kotinen, southern Finland). During summer and winter stratification, the highest methanotrophic activities were in the metalimnion, where oxygen concentration was <6 mmol m^{-3}. During an incomplete spring turnover and summer stratification period, 3–5 times more CH_4 was consumed by methanotrophs in the water column than was released to the atmosphere. The highest CH_4 effluxes (1.2–5.1 mmol m^{-2} d^{-1}) to the atmosphere occurred during the autumnal turnover despite observed methanotrophic activity in the whole water column. In winter, the amount of CH_4 consumed by methanotrophs (0.20 mol CH_4 m^{-2} during 6.5 months) was of the same order of magnitude as that during the ice-free period (0.22 mol CH_4 m^{-2} during 5.5 months). Annually ~80% of CH_4 diffused from the sediment was consumed by methanotrophs in the water column, and only 20% (0.11 mol CH_4 m^{-2} yr^{-1}) was released to the atmosphere. During the ice-free period, bacterial production measured as [^{14}C]leucine uptake showed a bell-shaped relation to CH_4 concentration. The highest production was found in the metalimnion at CH_4 concentrations ranging from 5 to 10 mmol m^{-3}. During summer stratification, net production of methanotrophs corresponded to 23–47% of total bacterial production, but during the autumn turnover, this proportion was higher (27–81%), indicating that methanotrophs offer a potentially significant source of carbon to zooplankton in stratified humic lakes.

Most lakes worldwide are net heterotrophic, with community respiration exceeding primary production ($R > P$); thus, lakes are conduits of terrestrially fixed carbon to the atmosphere (Cole et al. 1994; Algesten et al. 2003). This is especially pronounced in the boreal area, where numerous small lakes with high concentrations of allochthonous dissolved organic matter are typical of flat landscapes with catchments dominated by forested and peatland areas (Salonen et al 1983; Kortelainen 1993). Because of the high concentrations of colored humic matter, small headwater lakes are often steeply stratified in both summer and winter, and anoxic conditions often prevail in the hypolimnion. In freshwater ecosystems, methane (CH_4) is the major terminal product of the anaerobic decomposition of organic matter in the absence of alternative electron acceptors (NO_3^-, Fe_3^+, and SO_4^{2-}; cf. Capone and Kiene 1988). Compared with atmospheric concentration, boreal lake waters are typically supersaturated with CH_4, and concentrations >1,000-fold saturation have been observed in the anoxic hypolimnion of stratified humic lakes (Kortelainen et al. 2000; Huttunen et al. 2002; Kankaala et al. 2005*a*).

In the lake water column, CH_4 can be oxidized to carbon dioxide (CO_2) in the presence of oxygen and also partly incorporated into cells by methanotrophic microbes (e.g., Hanson and Hanson 1996). In stratified eutrophic lakes, this process has been shown to be limited to a narrow metalimnetic zone at oxic–anoxic interfaces (Rudd et al. 1974; Fallon et al. 1980; Bastviken et al. 2003). However, the supersaturation level of CH_4 at lake surfaces indicates that some proportion of CH_4 escapes methanotrophy and will be released to the atmosphere, especially during spring and autumnal turnover periods (Michmerhuizen et al. 1996; Riera et al. 1999). In the atmosphere, CH_4 is a greenhouse gas contributing to global radiative forcing (Houghton et al. 2001). On the other hand, stable carbon isotope signatures of zooplankton have indicated that methanotrophs might be important compartments of the food web in humic lakes (Jones et al. 1999). Thus, we wanted to study simultaneously (1) the im-

[1] Corresponding author (paula.kankaala@helsinki.fi).

Acknowledgments

We thank Sari Koskinen and Pertti Saaristo for assistance in the field and with laboratory work, Roger Jones for critical reading and correction of the language of the manuscript, and two anonymous reviewers for improving the final version of the manuscript.

This study was funded by the Academy of Finland (project 50389 and 104438) and by the Helsinki University Environment Research Centre (HERC).

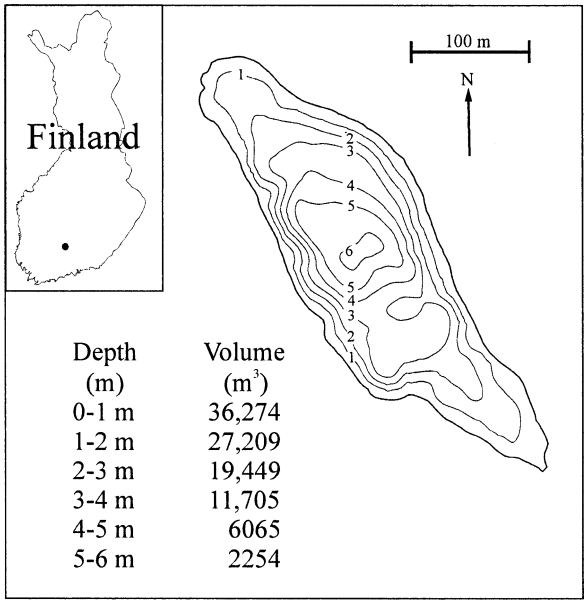

Fig. 1. Location of Lake Valkea-Kotinen (61°14′36.6″N, 25°4′25.7″E) in Finland and the map of the lake with depth zones for each meter. The volume of each zone is shown.

portance of a lake ecosystem in a boreal forested landscape as a source of CH_4 to the atmosphere, (2) the release of CH_4 from the lake surface to the atmosphere related to diffusion of CH_4 from the sediment and methanotrophic activity in the water column, and (3) the potential importance of methanotrophs in the food web of a humic lake. The study was carried out in a small humic headwater lake throughout a year from ice breakup in spring, weekly during the ice-free period, and for every 4–8 weeks during the ice-covered period in winter until the end of the next spring overturn.

Study area

Lake Valkea-Kotinen is a small (area 0.04 km^2, maximum depth 6.5 m, volume 103,000 m^3) headwater lake located in a boreal coniferous forest area in southern Finland (61°14′N, 25°4′E; Fig. 1). Because of its sheltered position and high concentration of allochthonous humic substances, the lake is normally steeply stratified with respect to temperature and oxygen. The spring turnover is usually short or incomplete, and anoxic conditions in the hypolimnion prevail usually from June until the autumn turnover in October. The lake is covered by ice for 5.5–6 months each year. In 1990–1996, the mean concentration of dissolved organic carbon varied between 800 and 1,000 mmol m^{-3}, and the mean concentration of total nitrogen and phosphorus in the epilimnion ranged from 31 to 40 and from 0.5 to 0.7 mmol m^{-3}, respectively. Secchi disc transparency is generally 1.4–1.6 m, and the euphotic zone reaches a depth of ~2 m. Annual primary production of phytoplankton varied between 2.1 and 3.2 mol C m^{-2} yr^{-1} during 1990–1996 (Keskitalo et al. 1998). The littoral zone of the lake is narrow, reaching to a depth of 1.5–1.6 m, and the vegetation is dominated by sparse stands of *Nuphar lutea* (L.) and submerged *Sphagnum* sp. (Keskitalo and Heitto 1996).

Methods

Methane concentration in water—Concentration of CH_4 in the water column was measured in 2002, once a month under the ice cover (January–April), weekly after melting of the ice (24 April) until formation of new ice cover (17 October), and thereafter once a month until the end of May 2003 at the deepest area of the lake from surface to 6 m depth at 1-m intervals. Duplicate samples of water (30 mL) were taken from a 30-cm-long Limnos tube sampler (volume 2 liters) into 60-mL polypropylene syringes (Terumo, Leuven, Belgium), which were closed with three-way stopcocks (Luer-lock, Codan Steritex). The syringes were kept in crushed ice (maximum 4 h) until the headspace of each was filled with 30 mL of N_2 gas, from which CH_4 concentration was measured according to McAuliffe (1971). During 2002, from January to September, an HP 5710A gas chromatograph (flame ionization detector [FID] 100°C, oven 40°C, HayeSepQ packed column with mesh 80/100, N_2 as a carrier gas) was used for CH_4 analyses. From October 2002, the analyses were made with an Agilent 6890N (Agilent Technologies) gas chromatograph (FID 205°C, oven 40°C, CarbonPlot capillary column, He as the carrier gas). The samples were injected through a 0.5-mL loop in a VALCO 10-port (or 1-mL loop in a 4-port) valve (VICI). Both instruments were calibrated with CH_4 calibration gases (1.878 and 50.1 ppm, AGA). The CH_4 concentration in the water was calculated as described by Huttunen et al. (2001). In the middle of the lake, the coefficient of variation of CH_4 concentration between replicate samples was generally <10%, and in the surface water samples (0–30 cm) taken from a transect from the shoreline to the middle of the lake (n = 4), the coefficient of variation was <15%.

During sampling, temperature and oxygen concentration was measured at 1-m intervals from surface to 6 m depth with a combined probe (Yellow Springs Instruments YSI 55, accuracy ±0.3°C, ±9 μmol O_2).

Methanotrophy—Methanotrophic activity was measured weekly from 12 June to 16 October 2002 and five times during the following winter and spring. Lake water from depths of 0, 2, 4, and 6 m taken with the Limnos sampler was transferred into dark 2-liter bottles, which were carefully flushed with water from the sampling depth before filling, and then closed with glass stoppers to avoid air headspace. In the laboratory, CH_4 oxidation was measured as a linear decrease of CH_4 concentration in sterile glass syringes (SAMCO Interchangeable, S Murray Co., 50 mL volume). Ten syringes for every sampling depth were filled half full (25 mL) with the lake water, carefully avoiding air bubbles, and closed with three-way stopcocks, and the connection between the plunger and the syringe was covered by Parafilm (American National Can). For every depth, the concentration of CH_4 was analyzed from three syringes immediately after filling (t_0). The other syringes were incubated in darkness in temperatures simulating those measured in the field (±2°C), and their CH_4 concentration was measured after 4, 8, and 24 h of incubation. Only those time series with a significant linear decrease of CH_4 ($p < 0.05$) were accepted as results of methanotrophic activity, whereas unchanged CH_4 concen-

tration during 24 h indicated that no methanotrophic activity had occurred.

Potential CH_4 limitation of methanotrophs in the epilimnion was estimated at 2 m depth. Five milliliters of CH_4 gas (99.5% AGA) was gently injected through a silicone tube into another dark 2-liter glass-stoppered bottle filled with water from 2 m depth, and the bottle was gently mixed. With this treatment, the CH_4 concentration was increased from 0.52 ± 0.76 to 2.40 ± 0.87 mmol m^{-3}. The linear decrease of CH_4 was then measured in glass syringes as described above.

A test of gas-tightness of the syringes was made in June 2002 with water taken from 0 and 4 m depth. Twelve syringes were filled half full with lake water from each depth as described previously. An amount of 0.5 mL of difluoromethane (DFM, concentration 98%, Lancaster Synthesis), a specific inhibitor of methanotrophs (Miller et al. 1998), was added into six syringes through the three-way stopcocks, whereas the other six were left without DFM addition. In three syringes of both treatments, CH_4 concentration was analyzed immediately (t_0), and in the other syringes 24 h later (t_{24}). At 0 m depth, the CH_4 concentration did not change significantly during 24 h in either syringes with DFM added or omitted ($p > 0.06$), indicating that no methanotrophic activity occurred in samples from that depth. At 4 m depth, no methanotrophic activity was observed in the syringes with DFM added (CH_4 concentration in $t_0 = 10.03 \pm 2.14$ and $t_{24} = 11.05 \pm 0.54$ mmol m^{-3}, $t = -1.357$, $p = 0.347$), whereas in syringes without DFM, the decrease in CH_4 concentration was significant ($t_0 = 13.16 \pm 0.33$, $t_{24} = 7.81 \pm 0.06$ mmol m^{-3}, $t = 27.35$, $p < 0.001$). Thus, these results show that syringes were gas tight, and the results obtained can be considered reliable.

An independent whole-lake estimate of CH_4 oxidation was derived from estimation of turbulent diffusion (TD) of CH_4 across the concentration gradient in the water column and comparing predicted and observed concentrations (mmol m^{-3}) of CH_4 in successive weekly samplings in the water column at each meter during the ice-free period (the TD method). The vertical diffusion coefficients K (m^2 d^{-1}) were estimated by the MyLake model (Saloranta and Andersen 2004),

$$K = a_k(N^2)^{-0.43}$$

where N^2 is the stability (Brunt-Väisälä) frequency

$$N^2 = \frac{g}{\rho_w}\frac{\partial \rho_w}{\partial z}\ (s^{-2})$$

(Hondzo and Stefan 1993), g is the gravitational constant, ρ_w is the density of water, and a_k is parameterized by lake surface area A_s (km^2). The default parameterization, $a_k = 0.00706(A_s)^{0.56}$ during the ice-free period, was adopted from Hondzo and Stefan (1993), who used temperature profiles from a Minnesota lake database to derive parameterizations for hypolimnetic eddy diffusivity. A minimum possible stability frequency, which sets the upper limit for K, was a default value $N^2_{min} = 7.0 \times 10^{-5}$ s^{-2} (Hondzo and Stefan 1993). The density profile from the previous model time step was used to calculate the profile of K for the present time step. Diffusion of CH_4 (D_{CH4} mmol m^{-2} d^{-1}) from the sediment upward in the water column in successive weekly samplings at each meter from the lower layer (z_{m-1}) to depth z_m ($\Delta z = z_{m-1} - z_m$) was calculated by multiplying K by the concentration gradient of CH_4 (mmol m^{-3}) at 1-m intervals at time t (Eq. 3).

$$D_{CH_4}(t, z_m) = \frac{K \times [CH_4(t, z_{m-1}) - CH_4(t, z_m)]}{\Delta z}$$

Predicted CH_4 concentration at time $t + \Delta t$ at depth z_m was calculated as in Eq. 4.

$$CH_4(t + \Delta t, z_m) = CH_4(t, z_m) + \frac{D_{CH_4}(t, z_{m-1}) - D_{CH_4}(t, z_m)}{\Delta z}\Delta t$$

Thus, CH_4 oxidized at z_m between successive samplings ($\Delta t = t_2 - t_1$) was estimated as a difference between predicted and observed CH_4 concentration (Eq. 5).

$$CH_4 \text{ oxidized}(t + \Delta t, z_m) = \frac{CH_4(t + \Delta t, z_m) - CH_4(t, z_m)}{\Delta t}$$

Concentration gradients above the sediment were calculated by assuming that the CH_4 concentrations measured at 6 m depth (0.5 m above sediment surface in the deepest area) were valid for the whole anoxic bottom below 3 m depth. The obtained results of CH_4 oxidation (mmol m^{-3} d^{-1}) were weighted by the volume of each 1-m layer and divided by the surface area of the lake to obtain results (mmol m^{-2} d^{-1}).

Net production of methanotrophs was estimated assuming bacterial growth efficiency ranging between 25% and 50%. This range was obtained from the average growth efficiency values for boreal humic lakes (Tulonen 2004) and lakes in general (del Giorgio and Cole 1998) and from the results of Rudd and Hamilton (1978) for methanotrophs in a Canadian eutrophic shield lake.

Heterotrophic bacterial production—Water for bacterial production measurements was sampled once a week from June to October at 1-m intervals with the Limnos tube sampler and transferred into sterile 500-mL cartons. Production measurements were carried out in vitro in the laboratory immediately after sampling. Triplicate samples of 5 mL in 20-mL preignited (450°C, 4 h) glass vials were incubated at 15°C in a water bath for 60 min with 40 nM of [^{14}C]leucine (specific activity 11.3 GBq $mmol^{-1}$, Amersham Biosciences). Glutardialdehyde-killed controls were run in parallel. Incubations were stopped with ice-cold 50% trichloroacetic acid at a final concentration of 5%, after which samples were cooled in ice for 15 min and filtered onto 0.2-μm cellulose nitrate filters (Whatman International Ltd.). The filters were rinsed with 0.5 mL of ice-cold 5% trichloroacetic acid and distilled water and dissolved in scintillation vials with 0.25 mL of ethylenglycolmonomethylether and 9 mL of Opti-Phase3 scintillation liquid for 48 h before being radioassayed with a Wallac 1409 (Wallac Oy) liquid scintillation counter. Leucine incorporation rates were converted to carbon production with a factor of 7.71×10^{15} μm^3 mol^{-1} and a biovolume-to-carbon conversion factor of 0.36 pg C μm^{-3} (Tulonen 1993). In July 2003, bacterial production rates

measured in vitro were twice compared with those measured in situ by incubating vials at respective sampling depths (Peltomaa, Huotari, and Ojala unpubl. data). From surface to 5 m depth, the incubation did not affect bacterial production rates significantly ($p > 0.2$). Only at the deepest layer (6 m) were bacterial production rates measured in vitro (0.48 $\pm$ 0.08 and 0.40 $\pm$ 0.11 mmol C m^{-3} d^{-1}) significantly higher ($p < 0.05$) than those measured in situ (0.23 $\pm$ 0.10 and 0.16 $\pm$ 0.04 mmol C m^{-3} d^{-1}).

CH_4 efflux to the atmosphere—During the ice-free period (29 April–16 October), CH_4 efflux from water surface to atmosphere was measured in the middle of the lake weekly at 9:00–11:00 h (Greenwich mean time + 2 h) with a static chamber technique. Air samples were transferred from three floating chambers (volume 5.8 liters, height 0.125 m) made of clear acrylic plastic into 60-mL polypropylene syringes at 5-min intervals for 30 min. Air temperature in the chambers during incubation was measured with NTC thermistors. Concentration of CH_4 was measured by gas chromatography (see above) within 4 h after sampling. CH_4 efflux was measured as a linear increase ($p < 0.05$) in CH_4 over time. The results (mmol m^{-2} h^{-1}) were calculated according to the ideal gas law, and assuming no diurnal variation of CH_4 efflux, the results were then calculated (mmol m^{-2} d^{-1}).

Another estimate of CH_4 efflux during the ice-free period was calculated with the boundary layer diffusion equations presented by Kling et al. (1992) and Phelps et al. (1998),

$$\text{CH}_4 \text{ efflux} = \frac{D_b}{z_b} \times (C_{sur} - C_{eq})$$

where z_b is the thickness of the boundary layer, C_{sur} is the concentration of CH_4 at 0–30 cm depth and C_{eq} is the concentration of CH_4 in equilibrium with air. The values of C_{eq} varied between 2.6 and 3.7 nmol, calculated with Henry's law constants for surface temperatures (Lide and Fredikse 1995) assuming a stable atmospheric CH_4 concentration of 1.745 ppm (Houghton et al. 2001). The diffusion coefficient (D_b, cm^2 s^{-1}) and z_b (μm) were calculated as in Eq. 7,

$$D_b = [1.33 + (0.055 \times T)] \times 10^{-5} \quad \text{and}$$

$$z_b = 10^{(2.56-(0.133\times ws))}$$

where T is water temperature (°C) at the surface and ws is wind speed at 10 m height (m s^{-1}). The wind speed (1 m above the lake surface) was obtained from a weather station established in the middle of the lake from 7 June 2002 onward. For the period from late April to early June 2002, the wind speed was assumed to be the mean value from 7 to 30 June 2002 (0.9 m s^{-1}). A factor of 1.22 was applied to convert wind speed at 1 m above the lake surface to that at 10 m height (Crusius and Wannikhof 2003).

Results

Lake stratification and CH_4 concentration—The water column of Lake Valkea-Kotinen was steeply stratified, especially during the ice-free period from late May to mid-September, a pattern that is observed annually in this lake (Keskitalo et al. 1998). The modeled turbulent diffusion coefficient K (m^2 d^{-1}) values during this period varied in the hypolimnion (3–6 m) between 0.019 and 0.073 and in the epilimnion (0–1.5 m) between 0.011 and 0.073, but the minimum occurred at the metalimnetic depth of 2 m, where it varied between 0.007 and 0.020 (Fig. 2). During the turnover periods, K values from all depths peaked at 0.073.

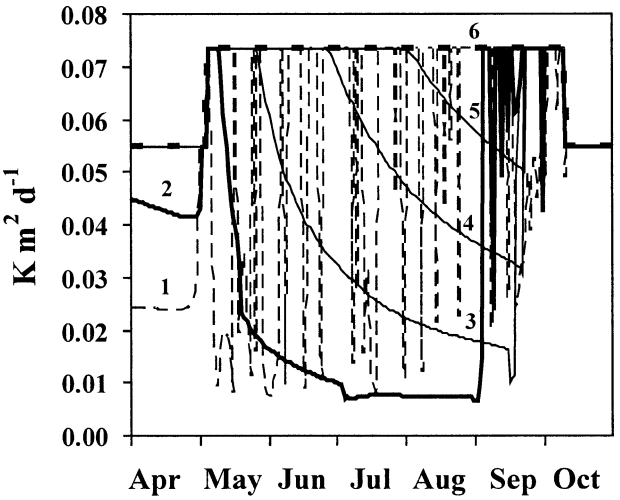

Fig. 2. Modeled turbulent diffusion coefficients in Lake Valkea-Kotinen from April to October 2002 at depths of 1–6 m.

At the lake surface (0–0.3 m) the CH_4 concentration ranged from 0.005 mmol CH_4 m^{-3} in early winter to 3.0 mmol CH_4 m^{-3} at the onset of autumnal turnover, but compared with the atmospheric concentration, lake surface was always supersaturated with CH_4. During the summer stratification period, oxygen concentration was at the minimum detection level (6 mmol m^{-3}) at 3 m depth in late June and at 2 m depth in late August (Fig. 3). The concentration of CH_4 in the hypolimnion increased in parallel; the maximum concentration reached 236 mmol m^{-3} at 6 m depth in mid-September. At that time, CH_4 storage in the water column was at the maximum (1,890 mol CH_4 $lake^{-1}$). The cooling of water masses and breakdown of stratification began in September, and the autumnal turnover of water masses was complete by mid-October. Because of ice formation around 3 weeks earlier in 2002 (17 October) than in the previous autumn, the period of anoxia near the bottom was longer and the accumulation of CH_4 in the water column was greater during the winter 2002–2003 compared with that during the previous winter (maximum concentrations at 6 m depth, 184 and 115 mmol m^{-3}, respectively). Accumulated CH_4 storage below the ice cover both winters (340 and 240 mol CH_4 $lake^{-1}$ in 2002–2003 and 2001–2002, respectively) was <20% of that accumulated during the summer stratification. The greater CH_4 accumulation in the hypolimnion during summer stratification than during winter ice cover was observed also during the years 2000–2001 (data not shown). Spring turnover during both study years was incomplete, and the oxygen concentration near the bottom (6 m) did not increase above the detection limit.

Methanotrophic activity and CH_4 diffusion in the water column—The highest methanotrophic activities were always

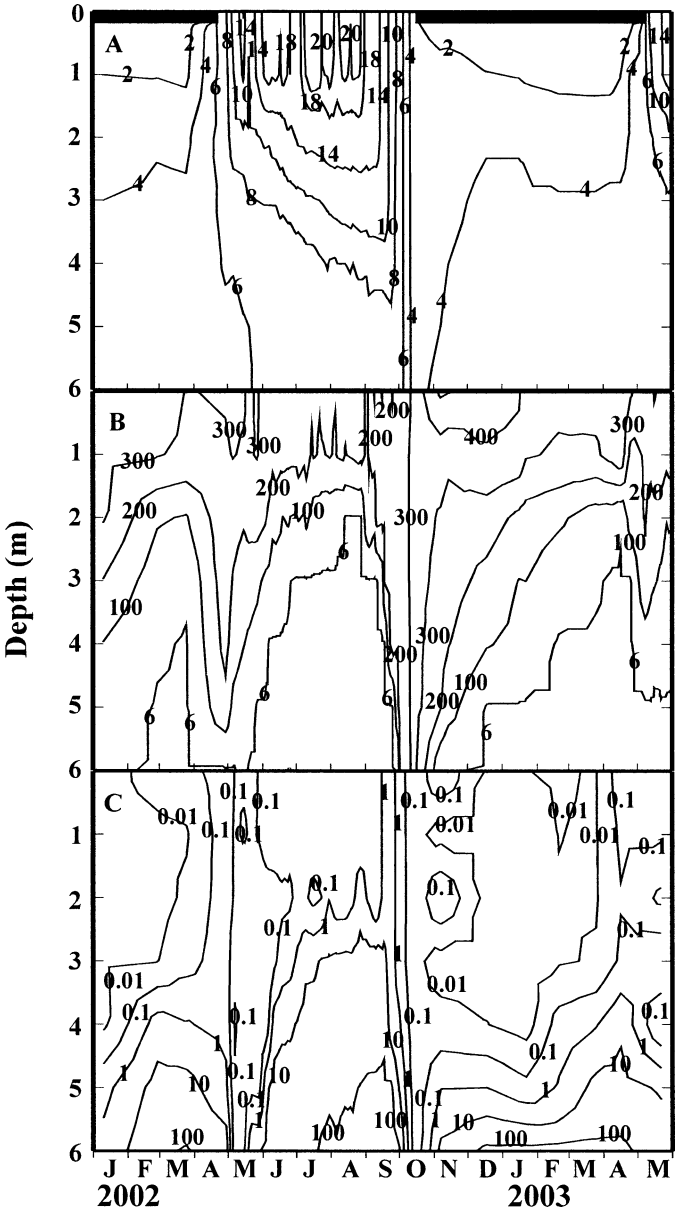

Fig. 3. (A) Temperature (°C), (B) oxygen (mmol O_2 m^{-3}), and (C) methane concentration (mmol CH_4 m^{-3}) in the water column of Lake Valkea-Kotinen from January 2002 to May 2003. Black horizontal bar on the top denotes ice cover.

measured at oxic–anoxic interfaces (<6 mmol O_2 m^{-3}; Fig. 4). The highest activities were measured at 6 m depth after the incomplete spring turnover (6–18 mmol CH_4 m^{-3} d^{-1}), but during the summer and winter stratification periods, no activity was measured at that depth, presumably because of oxygen limitation. At 4 m depth, methanotrophic activity was observed from June to mid-July and again from the onset of autumnal turnover in September to mid-October and during the next winter and spring from March to May.

In the epilimnion, methanotrophic activity was measured at 2 m depth from late June to mid-October (0.04–1.8 mmol CH_4 m^{-3} d^{-1}) and at the surface water layer (0–0.3 m) from July to mid-October (0.02–1.3 mmol CH_4 m^{-3} d^{-1}). At these

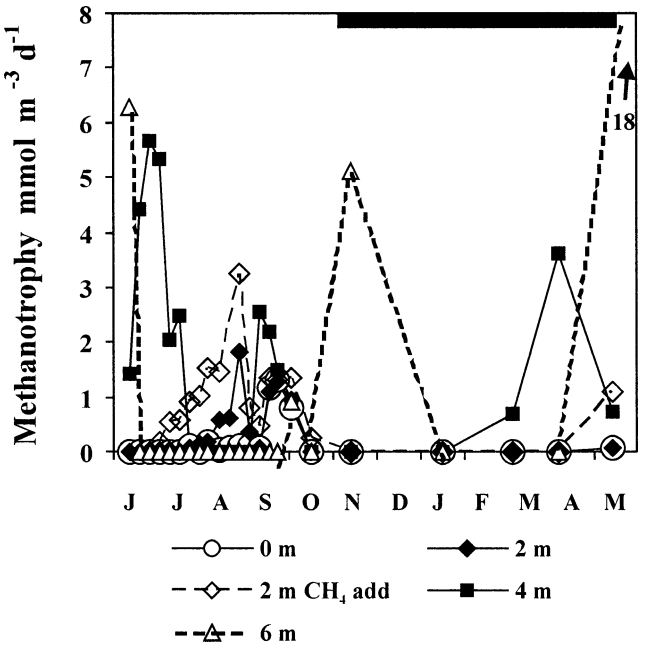

Fig. 4. Methanotrophy (mmol CH_4 m^{-3} d^{-1}) in the water of Lake Valkea-Kotinen measured from June 2002 until May 2003 at 0, 2, 4, and 6 m depth. At 2 m depth, results after CH_4 addition to the water samples are also given (see *Methods* for details). Black horizontal bar on the top denotes ice cover.

depths, methanotrophic activity was limited by CH_4 availability. This was demonstrated by a very significant linear correlation between the measured activity and CH_4 concentration at the onset of methanotrophic activity measurement (t_0; Fig. 5). Moreover, the addition of CH_4 into water from 2 m depth caused a 2–17-fold increase of methanotrophic activity during summer and >20-fold increase after autumn

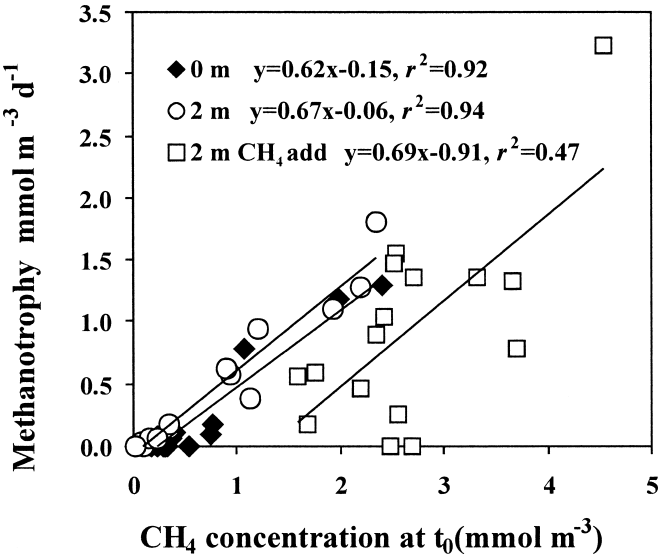

Fig. 5. Relationship between measured methanotrophic activity during the ice-free period (from mid-June to mid-October) at 0 and 2 m depths and at 2 m with CH_4 addition, and the CH_4 concentration at the beginning of measurement (t_0).

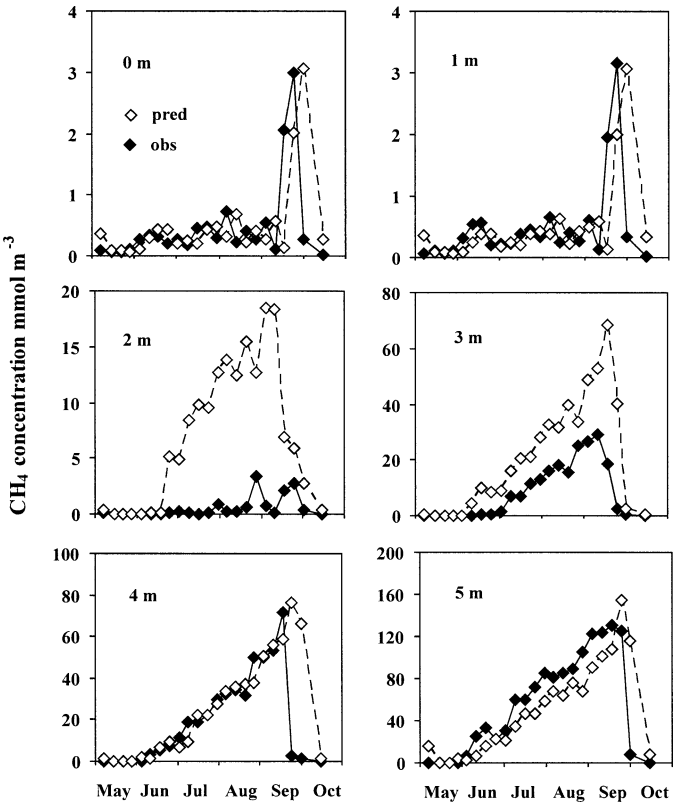

Fig. 6. Predicted CH_4 concentration, calculated as amount of CH_4 diffused upward in the water column and assuming no CH_4 oxidation activity (see *Methods*), and observed CH_4 concentration during the ice-free period at 0–5 m depths in Lake Valkea-Kotinen. Note different scales for CH_4 concentration.

and spring turnover periods compared with the activities measured at in situ concentrations (Fig. 4). In most of these experiments, methanotrophs were evidently still limited by CH_4 because there was also a linear correlation between t_0 CH_4 concentration and methanotrophic activity. In winter, no methanotrophic activity was measured at 0 and 2 m, and activity was not stimulated by the addition of CH_4.

When considering the whole water column during the summer stratification period, the difference between predicted (i.e., potentially transported from deeper layers by turbulent diffusion) and observed CH_4 concentration was greatest at the depths of 2 and 3 m (Fig. 6), indicating that methanotrophic activity was highest between the depths of 2 and 4 m. Despite the lowest modeled vertical diffusion rates occurring at 2 m depth in summer, the estimate of turbulent CH_4 diffusion from 3 to 2 m depth exceeded the measured methanotrophy in the epilimnion integrated over the 0–2-m layer (Fig. 7).

Heterotrophic bacterial production—From 7 June to 16 October, the heterotrophic bacterial production (HBP) varied between 0.1 and 1.8 mmol C m^{-3} d^{-1} (Fig. 8). The greatest production was at 3 m depth (mean ± SD 0.99 ± 0.45 mmol C m^{-3} d^{-1}), where it differed significantly from the other depths (Mann–Whitney *U*-test, $U > 190$, $p < 0.005$, $n = 17$). When all measurements of HBP at each depth during

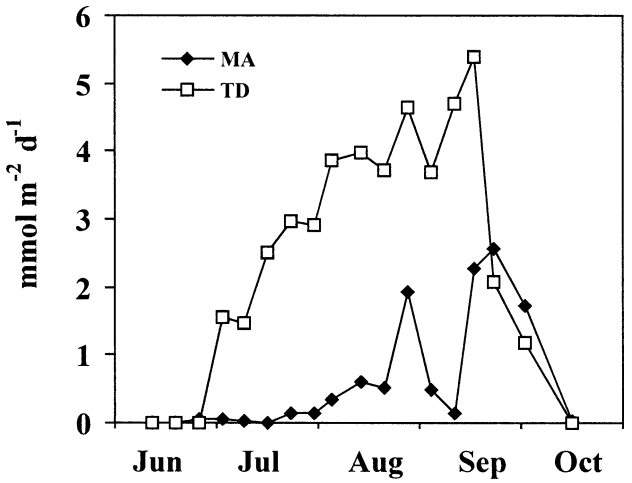

Fig. 7. Measured methanotrophic activity (MA, mmol CH_4 m^{-2} d^{-1}), integrated over the 0–2 m layer, and estimated turbulent diffusion (TD) of CH_4 from 3 to 2 m depth during the ice-free period of 2002 in Lake Valkea-Kotinen.

the ice-free period was plotted against CH_4 concentration, the relationship was bell shaped (log–log scale), with the highest activities occurring at concentrations of 5–10 mmol CH_4 m^{-3} (Fig. 9A).

The ratio between methanotrophic activity and bacterial production (MA : HBP) was calculated for those depths with parallel measurements within 24 h (i.e., 0, 2, 4, and 6 m) during June–October. This ratio varied between 0.01 and 10.4 and was lowest after the autumnal turnover of water in mid-October and highest at 6 m depth in June after the incomplete spring turnover. The MA : HBP ratio was very significantly related to CH_4 concentration (Fig. 9B). [^{14}C]Leucine uptake into bacterial cells probably reflects the net production by bacteria, whereas the methanotrophic activity, measured as consumption of CH_4 over time, probably

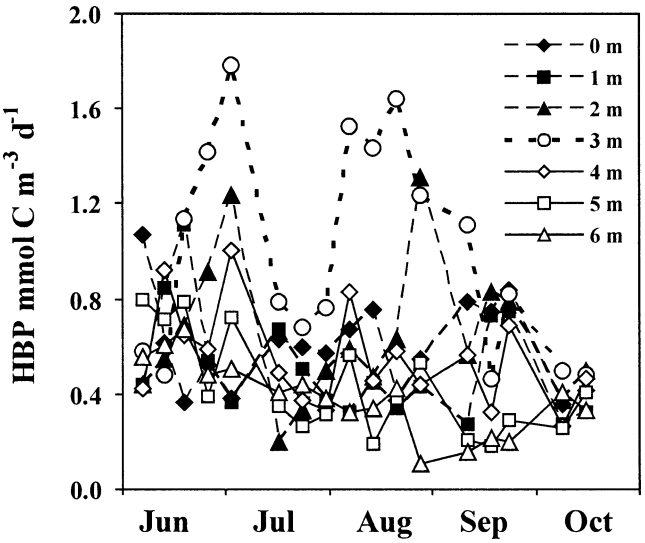

Fig. 8. Heterotrophic bacterial production (HBP, mmol C m^{-3} d^{-1}) measured with [^{14}C]leucine uptake method at 0–6 m depths in June–October in Lake Valkea-Kotinen.

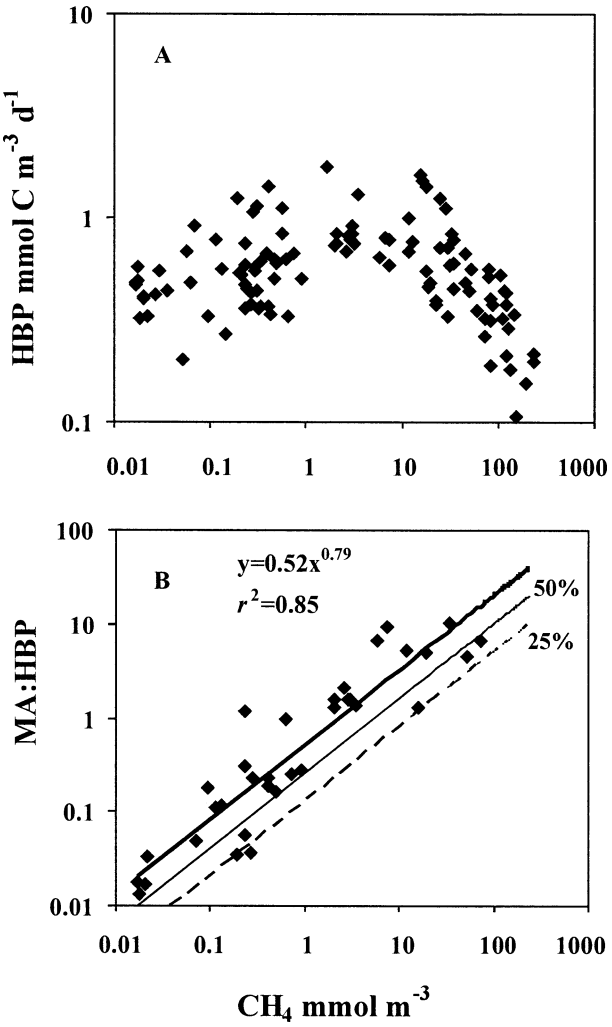

Fig. 9. (A) Heterotrophic bacterial production (HBP) related to CH_4 concentration in June–October; (B) ratio between methanotrophic activity and heterotrophic bacterial production (MA : HBP) related to CH_4 concentration. The lines representing the relationship of 25% and 50% growth efficiency of methanotrophs are also given.

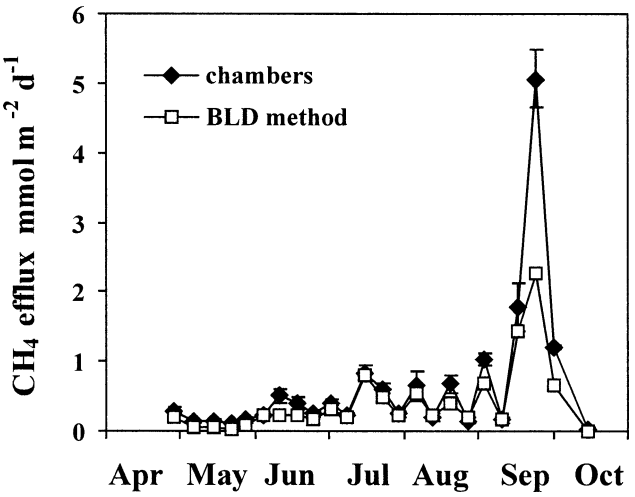

Fig. 10. Efflux of CH_4 (mmol CH_4 m^{-2} d^{-1}) from the surface of Lake Valkea-Kotinen during the ice-free period of 2002 measured with a closed-chamber technique and a boundary layer diffusion (BLD) method.

reflects the gross production of methanotrophs. Thus, assuming that the net growth efficiency of methanotrophs varies between 25% and 50%, we found that at CH_4 concentrations ranging from ~5 to 10 mmol m^{-3}, MA : HBP is ~1, which suggest that all HPB derived from methanotrophs in that range.

CH_4 efflux—During the ice-free period, CH_4 was continuously released from the lake surface to the atmosphere (Fig. 10). From late April to late August, the CH_4 efflux rate varied between 0.1 and 0.8 mmol CH_4 m^{-2} d^{-1}, but the highest efflux rates (1.2–5.1 mmol CH_4 m^{-2} d^{-1}) were measured from mid-September to early October during the autumnal turnover of water masses. Overall, the efflux rates estimated with the boundary layer diffusion method were very significantly correlated with the chamber measurements (r^2 = 0.91), but the peak values, especially during the autumnal turnover, were only about half of those with the chamber measurements. During the study period, the wind speed at this sheltered lake was low; the daily mean at 1 m above the surface was 1.0 m s^{-1}. Thus, the estimated wind speed at 10 m was ~1.22 m s^{-1} and it never exceeded 3.7 m s^{-1}.

Methane efflux by ebullition from the sediment was probably insignificant in this lake because no gas bubbles were trapped into subsurface funnels (n = 8) during a 3-week period in July 2003 (Huotari unpubl. data).

Annual CH_4 budget—The results of CH_4 oxidation in the water column of Valkea-Kotinen during the whole ice-free period, on the basis of direct weekly measurements and those estimated by the TD method, were very similar (Table 1), although the former method gave a 15% higher estimate for the stratification period and 30% lower estimate for the autumnal turnover than the latter method. The estimate of efflux on the basis of boundary layer diffusion was about one-third lower than that measured with the chamber technique during the ice-free period. However, considering the methods used in the oxidation and efflux estimates, the results show that during the summer stratification period, about three to five times more CH_4 was consumed in the water column than was released into the atmosphere. During the autumnal turnover, the efflux measured by the chamber technique was on the same order as methanotrophic activity. During the winter ice-covered period, total CH_4 consumption by methanotrophs in the water column was almost as high as during the ice-free period, although the daily mean values were then lower. In spring, CH_4 oxidation in the water column was higher than the efflux to the atmosphere. On an annual basis, 21% (0.11 mol m^{-2} yr^{-1}) of the CH_4 diffused from the sediment was released into the atmosphere and 79% (0.42 mol m^{-2} yr^{-1}) was consumed in the water column by methanotrophs.

By comparing the estimated range of net production by methanotrophs with bacterial production measured with the [^{14}C]leucine incorporation technique, we can roughly estimate that during summer stratification, net production of methanotrophs corresponded to 23–47% of the total bacterial

Table 1. Methanotrophic activity, measured as a direct consumption of CH_4 and estimated by turbulent diffusion (TD) of CH_4 through the water column (TD method), as well as efflux of CH_4, measured with the chamber technique and estimated by the boundary layer diffusion equation (BLD), from late April 2002 to the end of May 2003 in Lake Valkea-Kotinen. Heterotrophic bacterial production (HBP) was measured only for the summer stratification and autumn turnover periods. A range for net production by methanotrophs (NPM) was obtained assuming growth efficiency (GE) varying from 25% to 50% of methanotrophic activity. The values in bold are rates (mmol m^{-2}) integrated over the respective periods; the values in parentheses are measured daily mean ± SD values (mmol m^{-2} d^{-1}) for the respective periods.

Method	Days (*n*)	Methanotrophic activity		CH_4 efflux		HBP of [^{14}C]leucine	NPM (GE 0.25–0.5)
		Measured	TD	Chamber	BLD		
Spring (29 Apr–31 May 2002)	33	**nd**	**10** (0.5±1.0)	**5** (0.2±0.1)	**2** (0.1±0.1)	**nd**	**3–5**
Summer stratification (1 Jun–17 Sep 2002)	109	**161** (1.3±0.9)	**140** (1.0±0.9)	**52** (0.4±0.3)	**41** (0.3±0.2)	**170** (1.6±0.3)	**40–80**
Autumn turnover (18 Sep–16 Oct 2002)	29	**48** (2.2±1.5)	**68** (2.9±2.3)	**54** (2.0±2.2)	**29** (1.1±0.9)	**37** (1.3±0.5)	**10–30**
Whole ice-free period (29 Apr–16 Oct 2002)	171	**219***	**218**	**111**	**72**		**53–115**
Winter ice-covered period (17 Oct 2002–7 May 2003)	203	**195** (0.7±0.7)	**nd**	**0**†	**0**†	**nd**	**49–98**
Spring (8–31 May 2003)	24	**47** (2.0±0.4)	**nd**	**nd**	**3** (0.1±0.1)	**nd**	**12–24**
Whole year (29 Apr 2002–28 Apr 2003)	365	**408**		**111**	**72**		**102–204**

nd, not determined.
* For the spring period (29 Apr 2002–31 May 2002), the direct measurement of methanotrophic activity was assumed to be the same as that estimated by the TD method.
† Assumed to be 0, although not measured.

production, and during autumnal turnover, this proportion was 27–81% (Table 1).

Discussion

During the whole ice-free period, the surface layer of Lake Valkea-Kotinen was supersaturated by CH_4, and the lake released CH_4 to the atmosphere. The estimate obtained from floating chamber measurements (0.11 mol CH_4 m^{-2} yr^{-1}) can be regarded as more reliable than that calculated by the boundary layer diffusion equation (0.07 mol CH_4 m^{-2} yr^{-1}) because the thickness of the boundary layer is related to wind speed in the equation applied (Phelps et al. 1998). For the whole study period, the daily mean wind speed 10 m above the surface of this sheltered lake was <3.7 m s^{-1}. Crusius and Wannikhof (2003) showed that at wind speeds of >3.7 m s^{-1} at 10 m height, gas transfer velocity was linearly related to wind speed. At lower wind speeds, gas exchange across air–water interfaces is independent of wind speed and influenced by other processes, such as convective cooling or precipitation (Cole and Caraco 1998; Crusius and Wannikhof 2003), which might be true also in Lake Valkea-Kotinen.

CH_4 efflux during the ice-free period from the pelagic zone of Valkea-Kotinen (0.11 mol CH_4 m^{-2} yr^{-1}) was lower than has been measured from natural boreal peatlands (ombrogenous bogs 0.5 and minerogenous fens 1.2 mol CH_4 m^{-2} yr^{-1}; Nykänen et al. 1998) and vegetated littoral areas of mesoeutrophic lakes from the same region (0.2–2.7 mol CH_4 m^{-2} yr^{-1}; Hyvönen et al. 1998; Kankaala et al. 2005*b*). In peatlands and continuously inundated littoral areas, the seasonal maximum of CH_4 efflux coincides with the period of highest soil/sediment temperature, plant productivity, or both; thus, it is more related to methanogenic than methanotrophic activity in the soil/sediment (e.g., van der Nat and Middelburg 1998; Kankaala and Bergström 2003; Werner et al. 2003). Our study in Lake Valkea-Kotinen demonstrates that in a stratified lake, CH_4 efflux is related to methanotrophic activity, CH_4 accumulation in the hypolimnion, and physical mixing in the water column.

In lakes with an anoxic hypolimnion in winter, high CH_4 effluxes to the atmosphere can occur during a short period in spring after melting of the ice cover (Michmerhuizen et al. 1996; Phelps et al. 1998). However, in spring in Lake Valkea-Kotinen, more CH_4 was oxidized in the water column than released to the atmosphere, and the daily CH_4 effluxes measured after melting of ice were on the same order of magnitude as those measured during the summer stratification period. The highest CH_4 effluxes from Valkea-Kotinen were during the autumnal turnover period because of a greater accumulation of CH_4 in the hypolimnion during summer than winter and also to a shorter period of turbulent mixing during the spring turnover compared with that in autumn. Very short or incomplete spring turnover is a typical phenomenon in small sheltered boreal humic lakes because stained humic substances rapidly absorb solar radiation and a lighter epilimnetic layer can be formed within a few days after melting of ice cover (Salonen et al. 1984; Keskitalo et al. 1998). Nevertheless, the mixing of the water column still

ensures enough oxygen for the bulk of CH_4 accumulated in winter to be oxidized in the water column rather than released to the atmosphere.

During the summer stratification period, three to five times more CH_4 was consumed by methanotrophs in the water column than was released to the atmosphere. The highest methanotrophic activities were observed at layers where the O_2 concentration was at the detection limit or below, typical of stratified lakes (Rudd et al. 1974; Harrits and Hanson 1980). Two independent methods were applied to determine methanotrophic activity within the water column: (1) direct measurements of methanotrophic activity carried out weekly with water sampled every second meter in the water column and (2) comparison of predicted CH_4 concentration (i.e., the amount of CH_4 transported by turbulent diffusion in the water column assuming no CH_4 oxidation activity occurred) with observed CH_4 concentration at each meter in weekly samples (TD method). These methods gave remarkably similar results, so the latter method involving less laboratory analysis can be successfully applied in stratified lakes for further study of the magnitude of CH_4 oxidation in annual budgets, whereas the direct measurement of methanotrophy provides more detailed information about the actual process.

Although the highest methanotrophic activities were observed at oxic–anoxic interfaces, CH_4 was also consumed in the epilimnion during the summer stratification period. There the methanotrophic activity was significantly correlated with the concentration of CH_4 and was stimulated by CH_4 addition, indicating that methanotrophic activity was limited by CH_4 availability. However, more CH_4 passed across the steepest density gradient from 3 to 2 m depth (see Fig. 7) than methanotrophs could utilize in the epilimnion at 0–2 m depth. This was also confirmed by the release of CH_4 throughout the ice-free period from the lake surface that was not completely used in the water column. At these low CH_4 concentrations, the kinetics of methane oxidation might have been reduced, or high oxygen concentration probably inhibited methanotrophy in the epilimnion. On the other hand, heavy grazing pressure by zooplankton on methanotrophic microbes also might have had some influence.

Convective cooling of water masses in autumn causes complete mixing of water masses in small boreal lakes like Lake Valkea-Kotinen. Despite efficient CH_4 oxidation in the whole water column, the highest CH_4 effluxes to atmosphere were then observed during a short period. The duration of the autumnal turnover period before freezing of the lake is critical to how well the whole water column is saturated by oxygen and whether anoxia, followed by CH_4 accumulation, will develop in bottom water layers in winter. In our study, we found that, despite lower temperatures in winter, methanotrophic activity at the oxic–anoxic interface was on the same order as in summer. Thus, the amount of annual CH_4 efflux is more influenced by the processes during the summer stratification period—diffusion of CH_4 from the sediment and its accumulation in the hypolimnion and oxidation in the water column—than those taking place in winter under ice cover.

During the summer stratification period in Lake Valkea-Kotinen, the highest bacterial production, measured with the [^{14}C]leucine uptake method, was measured at 3 m depth at the oxic–anoxic interface. This is in accordance with observations of McDonough et al. (1986) from a stratified lake with anoxic hypolimnion, in which the highest bacterial activities and incorporation of [^{3}H]thymidine and [^{3}H]leucine into protein were measured at the metalimnion at a depth at which oxygen concentration first becomes unmeasurable. In Lake Valkea-Kotinen, a bell-shaped relationship between bacterial production and CH_4 concentration at respective depths, reaching a maximum at 5–10 mmol CH_4 m^{-3}, strongly suggests that methanotrophs were mainly responsible for the bacterial production maximum at 3 m depth.

Bastviken et al. (2003) measured methanotrophic bacterial production as incorporation of $^{14}CH_4$ into bacterial cells in three boreal lakes during the summer stratification period (July or August) and in winter (March). They estimated that in summer, 0.5–10% of the total heterotrophic bacterial production, measured as [^{14}C]leucine uptake, was attributable to methanotrophs, but in winter, the proportion was higher; in one of the lakes, the production of methanotrophs corresponded to 68–120% of the bacterial production, but in the other two lakes, the proportion was between 3% and 39%. In their study, the growth efficiency of methanotrophs ranged from 6% to 80%. For a generalized estimate throughout a year, we assumed a narrower range of growth efficiency for methanotrophs of 25–50% (Rudd and Hamilton 1978; del Giorgio and Cole 1998). On the basis of these assumptions, net production of methanotrophs in Lake Valkea-Kotinen corresponded to 23–47% of the total bacterial production during the summer stratification period, and during autumnal turnover, this proportion was 27–81%. We did not measure bacterial production by [^{14}C]leucine uptake in winter, but because the observed methanotrophic activity was of the same order of magnitude, the role of methanotrophs in total bacterial production could not be lower in winter than in summer. In fact, the situation was probably opposite because algal primary production as a source of dissolved organic matter to bacteria was negligible in winter under severe light limitation beneath the ice and snow cover. During the ice-free period of 2002, the algal primary production of the lake was 1.1 mol C m^{-2} (Peltomaa, Huotari, and Ojala unpubl. data). The net production of methanotrophs (Table 1) was around 5–10% of this algal production. Thus, methanotrophs can potentially be a significant pathway of carbon from profundal sediment to the pelagic food web. The low carbon isotopic signatures ($\delta^{13}C < -30‰$) of zooplankton in humic lakes (Jones et al. 1999) support this conclusion.

References

ALGESTEN, G., S. SOBEK, A.-K. BERGSTRÖM, A. ÅGREN, L. TRANVIK, AND M. JANSSON. 2003. Role of lakes for organic carbon cycling in the boreal zone. Glob. Chang. Biol. **10:** 141–147; doi:10.1046/j.1529–8817.2003.00721.x.

BASTVIKEN, D., J. EJLERTSON, I. SUNDH, AND L. TRANVIK. 2003. Methane as a source of carbon and energy for lake pelagic food webs. Ecology **84:** 969–981.

CAPONE, D. G., AND R. P. KIENE. 1988. Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic carbon catabolism. Limnol. Oceanogr. **33:** 725–749.

COLE, J. J., AND N. F. CARACO. 1998. Atmospheric exchange of

carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF_6. Limnol. Oceanogr. **43:** 647–656.

———, ———, G. W. KLING, AND T. K. KRATZ. 1994. Carbon dioxide supersaturation in the surface waters of lakes. Science **265:** 1568–1570.

CRUSIUS, J., AND R. WANNIKHOF. 2003. Gas transfer velocities at low wind speed over a lake. Limnol. Oceanogr. **48:** 1010–1017.

DEL GIORGIO, P. A., AND J. J. COLE. 1998. Bacterial growth efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. **29:** 503–541.

FALLON, R. D., S. HARRITS, R. S. HANSON, AND T. D. BROCK. 1980. The role of methane in internal carbon cycling in Lake Mendota during summer stratification. Limnol. Oceanogr. **25:** 357–360.

HANSON, R. S., AND T. E. HANSON. 1996. Methanotrophic bacteria. Microbiol. Rev. **60:** 439–471.

HARRITS, S. M., AND R. S. HANSON. 1980. Stratification of aerobic methane-oxidizing organisms in Lake Mendota, Madison, Wisconsin. Limnol. Oceanogr. **25:** 412–421.

HONDZO, M., AND H. G. STEFAN. 1993. Lake water temperature simulation model. J. Hydraul. Eng. **119:** 1251–1273.

HOUGHTON, J. T., Y. DING, D. J. GRIGGS, M. NOGUER, P. J. VAN DER LINDEN, AND D. XIAOSU [EDS.]. 2001. Climate change 2001. The scientific basis. Cambridge Univ. Press.

HUTTUNEN, J. T., T. HAMMAR, J. ALM, J. SILVOLA, AND P. J. MARTIKAINEN. 2001. Greenhouse gases in non-oxygenated and artifically oxygenated eutrophied lakes during winter stratification. J. Environ. Qual. **30:** 387–394.

———, T. S. VÄISÄNEN, M. HEIKKINEN, S. HELLSTEN, H. NYKÄNEN, O. NENONEN, AND P. J. MARTIKAINEN. 2002. Exchange of CO_2, CH_4 and N_2O between the atmosphere and two northern boreal ponds with catchments dominated by peatlands or forests. Plant Soil **242:** 137–146.

HYVÖNEN, T., A. OJALA, P. KANKAALA, AND P. MARTIKAINEN. 1998. Methane release from stands of water horsetail (*Equisetum fluviatile)* in a boreal lake. Freshw. Biol. **40:** 275–284.

JONES, R. I., J. GREY, D. SLEEP, AND L. ARVOLA. 1999. Stable isotope analysis of zooplankton carbon nutrition in humic lakes. Oikos **86:** 97–104.

KANKAALA, P., AND I. BERGSTRÖM. 2004. Emission and oxidation of methane in *Equisetum fluviatile* stands growing on organic sediment and sand bottoms. Biogeochemistry **67:** 21–37.

———, J. HUOTARI, E. PELTOMAA, A. OJALA, AND L. ARVOLA. 2005*a.* Methanotrophic activity in relation to primary and bacterial production in a boreal humic lake. Verh. Internat. Verein. Limnol. **29:** 250–253.

———, T. KÄKI, S. MÄKELÄ, A. OJALA, H. PAJUNEN, AND L. ARVOLA. 2005*b*. Methane efflux in relation to plant biomass and sediment characteristics in stands of three common emergent macrophytes in boreal mesoeutrophic lakes. Glob. Chang. Biol. **11:** 145–153; doi:10.1111/j.365–2486.2004.00888x.

KESKITALO, J., AND L. HEITTO. 1996. Aquatic macrophytes in the acidified brown-water Lake Valkea-Kotinen, southern Finland. Lutukka **12:** 3–8. (In Finnish with English summary.)

———, K. SALONEN, AND A.-L. HOLOPAINEN. 1998. Long-term fluctuations in environmental conditions, plankton and macrophytes in a humic lake, Valkea-Kotinen. Boreal. Environ. Res. **3:** 251–262.

KLING, G. W., G. W. KIPPHUT, AND M. C. MILLER. 1992. The flux of CO_2 and CH_4 from lakes and rivers in arctic Alaska. Hydrobiologia **240:** 23–36.

KORTELAINEN, P. 1993. Content of organic carbon in Finnish lakes and its relationship to catchment characteristics. Can. J. Fish. Aquat. Sci. **50:** 1477–1483.

———, J. T. HUTTUNEN, T. VÄISÄNEN, T. MATTSSON, P. KARJALAINEN, AND P. J. MARTIKAINEN. 2000. CH_4, CO_2 and N_2O supersaturation in 12 Finnish lakes before and after ice-melt. Verh. Int. Verein. Limnol. **27:** 1410–1414.

LIDE, D. R., AND H. P. R. FREDIKSE [EDS.]. 1995. CRC handbook of chemistry and physics, 76th ed. CRC Press.

MCAULIFFE, C. C. 1971. GC determination of solutes by multiple phase equilibration. Chem. Technol. **1:** 46–51.

MCDONOUGH, R. J., R. W. SANDERS, K. G. PORTER, AND D. L. KIRCHMAN. 1986. Depth distribution of bacterial production in a stratified lake with an anoxic hypolimnion. Appl. Environ. Microbiol. **52:** 992–1000.

MICHMERHUIZEN, C. M., R. G. STRIEGL, AND M. E. MCDONALD. 1996. Potential methane emission from north-temperate lakes following ice melt. Limnol. Oceanogr. **41:** 985–991.

MILLER, L. G., C. SASSON, AND R. S. OREMLAND. 1998. Difluoromethane, a new and improved inhibitor of methanotrophy. Appl. Environ. Microbiol. **64:** 4357–4362.

NYKÄNEN, H., J. ALM, J. SILVOLA, K. TOLONEN, AND P. J. MARTIKAINEN. 1998. Methane fluxes on boreal peatlands of different fertility and the effect of long-term experimental lowering of the water table on flux rates. Glob. Biogeochem. Cycles **12:** 53–69.

PHELPS, A. R., K. M. PETERSON, AND M. O. JEFFRIES. 1998. Methane efflux from high-latitude lakes during spring ice melt. J. Geophys. Res. **103:** 29029–29036.

RIERA, J. L., J. E. SCHINDLER, AND T. K. KRATZ. 1999. Seasonal dynamics of carbon dioxide and methane in two clear-water and two bog lakes in northern Wisconsin, U.S.A. Can. J. Fish. Aquat. Sci. **56:** 265–274.

RUDD, J. W. M., AND R. D. HAMILTON. 1978. Methane cycling in a eutrophic shield lake and its effects on whole lake metabolism. Limnol. Oceanogr. **23:** 337–348.

———, ———, AND N. E. R. CAMPBELL. 1974. Measurement of microbial oxidation of methane in lake water. Limnol. Oceanogr. **19:** 519–524.

SALONEN, K., L. ARVOLA, AND M. RASK. 1984. Autumnal and vernal circulation of small forest lakes in southern Finland. Verh. Int. Verein. Limnol. **22:** 103–107.

———, K. KONONEN, AND L. ARVOLA. 1983. Respiration of plankton in two small, polyhumic lakes. Hydrobiologia **101:** 65–70.

SALORANTA, T., AND T. ANDERSEN. 2004. MyLake v. 1.1. Technical documentation & users guide. NIVA-report 4838. Norwegian Institute of Water Reserch.

TULONEN, T. 1993. Bacterial production in a mesohumic lake estimated from [^{14}C]leucine incorporation rate. Microb. Ecol. **26:** 201–217.

———. 2004. Role of allochthonous and autochthonous dissolved organic matter (DOM) as a carbon source for bacterioplankton in boreal humic lakes. Ph.D. thesis, Univ. of Helsinki.

VAN DER NAT F.-J.W.A., AND J. J. MIDDELBURG. 1998. Effects of two common macrophytes on methane dynamics in freshwater sediments. Biogeochemistry **43:** 79–104.

WERNER, C., K. DAVIS, P. BAKWIN, Y. CHUIXIANG, D. HURST, AND L. LOCK. 2003. Regional-scale measurements of CH_4 exchange from a tall tower over a mixed temperate/boreal lowland and wetland forest. Glob. Chang. Biol. **9:** 1251–1261.

Received: 26 April 2005
Accepted: 1 September 2005
Amended: 4 October 2005