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Research Article

Methane in an acidic bog lake: The influence of peat in the catchment on the biogeochemistry of methane

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Abstract. Methanogenesis, an anaerobic microbial process in sediments, was investigated in a naturally acidic bog lake, Grosse Fuchskuhle, in northeastern Germany. The lake was artificially divided into four sub-basins: two western basins receiving humic substances from an adjacent *Sphagnum* bog and two eastern basins isolated from this acidic inflow with installed curtains. Within the northeast (NE) and southwest (SW) basin compartments, one of each group were studied. In the peat influenced western basins the pH-values in the water columns were kept low and increased in the others. The stratification period increased in all four compartments from some weeks to some months, from April to October, with the development of anoxic hypolimnia in all compartments after the installation of the curtains. The layer with the most active methane production moved from a sediment depth of more than 20 cm before separation to close to the surface sediments in all compartments. Methanogenic microorganisms were found in the whole sediment core – from surface sediments to a depth of 25 cm. The proportion of methanogens was approximately 15% of total microbial cell numbers, which were approximately 2×10^6 cells/ml sediment. Oligonucleotide probes targeting nearly all families of the phylum Euryarchaeota were tested with fluorescence in-situ hybridization (FISH). In both basins, with and without the influence from peat, oligonucleotide probe (MSMX) targeting *Methanosarcinaceae* could be detected only as methanogens. The finding of only acetate-using methanogens by FISH indicated acetate as a major methanogenic substrate. Concentration profiles of CH_4 as a function of sediment depth were characterized in both basins by high concentrations in the top layers. Methane bubbles were released in the late summer in the eastern sub-basin only, likely from oversaturation in the surface sediments.

Key words. Methane; acidic lake; peat; methanogens; methanogenesis.

Introduction

Lake Grosse Fuchskuhle, a naturally acidic bog lake in northeastern Germany was chosen for whole-lake experiments (Kasprzak, 1993; Kasprzak et al., 1988; Koschel, 1995). The lake was divided by plastic curtains into four sub-basins, resulting in a restriction of the interactions between the lake and the adjacent peat to the western basins. Previous studies showed that the partitioning of this acidic lake has caused divergent developments within the resultant sub-basins, e.g., in phytoplankton primary production (Hehmann and Krienitz, 1996), structure of phytoplankton communities (Hehmann et al., 2001), zoo-

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plankton species composition (Kasprzak, pers. comm.), microbes and microbial activities in the water column (Babenzien and Babenzien, 1990; Šimek et al., 1998; Bittl and Babenzien, 1996), and water chemistry (Koschel, 1995). The latter study showed an increase in pH in the eastern sub-basins cut off from the peat. As a general outline of these intense studies conducted in Lake Grosse Fuchskuhle, it can be summarized that the NE sub-basin (chosen as an example of the less acidic ones) was characterized by higher biomass and activity throughout all trophic levels in the water column than the SW sub-basin (example of acidic basins).

The high oxygen demand of bacterial mineralisation and a lower oxygen input from the atmosphere, as a consequence of a stable stratification after separation, led to pronounced anoxic conditions in the hypolimnia. An oxygen free sediment-water interface may lead to a decrease in the nutrient (especially phosphorus)-retention capacity of the sediments and thus, to higher release rates of nutrients (e.g., Einsele, 1936; Caraco et al., 1993). Besides chemical reactions, microbial activities in the sediments, as methanogenesis, were the driving force for nutrient remobilization by the transformation of particulate materials into dissolved forms (Casper et al., 2000a). Due to increased nutrient release from the sediments the degree of bottom-up effects on the lake's food chain can drastically increase.

Only scattered information is available about the responses of sediment microorganisms to low pH in the water column (Baker et al., 1982; Rao et al., 1984; Naguib and Adams, 1996). Rao et al. (1984) described an impact on heterotrophic sediment bacteria when pH values in the sediment became lower than 5.5. Another effect of low pH in lake water is reported to be the increased accumulation of organic matter in the sediments (Rao et al., 1984). In a lake in northern Germany, Naguib and Adams (1996) found low methanogenic activities in sediments while pH in the water was low (3.3–3.8) and in sediments where pH reached values between 6–7. These findings were in agreement with previous results concerning methanogenic activities in the original Lake Grosse Fuchskuhle (Casper, 1992a, b) when low methane production rates were found in sediment depths of about 20 cm throughout the year. The divided lake opened new opportunities for studies on changing environments in the direction from acidic to neutralized conditions.

In Lake Grosse Fuchskuhle two main factors forcing the development of the compartments were defined: 1) the composition of DOC with an increased quantity in all sub-basins (Hehmann et al., 2001) but with pronounced differences in quality (Sachse et al., 2001), and 2) the long lasting period of anoxic conditions at the sediment-water interface in all basins.

The aim of this study was to determine if the differences developed between the compartments, caused by the different catchment areas and by the changed hydrophysical properties, influenced microbial methanogenic activity in the sediments. Molecular-biological investigations of the methanogens were also conducted to identify differences in the community structure after 11 years of separation. The influence of peat in the catchment was also investigated to assess its effect on sediment methane formation within the lake.

Material and methods

Study site

The study site was a small (1.5 ha) naturally acidic lake, Grosse Fuchskuhle, in a forested area at the western side of the Lake Stechlin area in northeastern Germany, about 100 km north of Berlin. Some general limnological parameters are summarized in Table 1. The lake is fed by ground water and precipitation (about 590 mm/y) (Richter, 1997; Casper and Koschel, 1995). The maximum and mean depths were 5.5 and 3.5 m, respectively; the sheltered location of this shallow lake allowed for thermal stratification of the water column during some weeks in early summer.

The lake was divided by plastic curtains in 1986 into two basins (east and west) and into four compartments by a second east-west separation in 1990. The catchment areas of these four basins differed strongly from each other; however, there were similarities with the eastern as well as the western sub-basins (Gross, 1999; Fig. 1). Within the northeast (NE) and southwest (SW) sub-basin compartments, one of each group was chosen for study. The western basins received allochthonic inputs from the bog, mainly humic acids, and the pH remained low at approximately 4.5 (Sachse et al., 2001). The pH increased up to 6.1 in the eastern compartments, which were cut off from the bog and the inflow of acidic substances. The sub-basins, each with an area of about 3,500 m^2, stratify each summer. A benthic O_2 consumption rate that exceeded the oxygen supply from the overlying water leads to the establishment of anoxic hypolimnia in all compartments, usually from May to September/October.

Table 1. Limnological parameters of the NE and SW basins of Lake Grosse Fuchskuhle and of the original lake (according to various authors).

Basins	NE	SW	Original lake
Area (m^2)	3,360	4,430	15,000
Volume (m^3)	11,300	9,700	53,000
Mean depth (m)	3.3	2.2	3.5
Catchment area (km^2)	<0.001	0.003	0.005
pH [a]	4.6–6.1	4.2–4.6	4.2–4.6
DOC (mg/L)	11.3 [b]	14.1 [b]	2.7 [a]

[a] In 0–2.5 m water layer; [b] in 4.0 m.

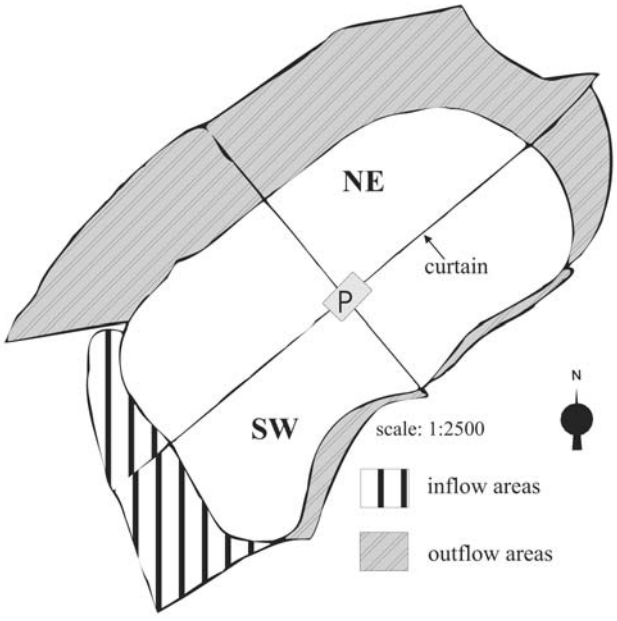

Figure 1. Map of Lake Grosse Fuchskuhle with catchment area (scale 1:2,500). The positions of the dividing curtains are given; the platform for sampling is indicated with P. The inflow areas are marked as black and white patterns, the outflow areas as a gray pattern; (redrawn and modified after Gross, 1999).

The basic limnology of Lake Grosse Fuchskuhle is described by Casper (1985), with microbiological aspects detailed in Babenzien and Babenzien (1990), Casper (1992a, b), and Šimek et al. (1998). Information about changes in the water column after basin division is provided in Koschel (1995), Koschel et al. (1995), Kasprzak (1993), and Hehmann and Krienitz (1996).

Water and sediment sampling

All samples were taken from a platform in the center of the lake (Fig. 1) from where the deepest points of all four compartments can nearly be reached. The water columns were sampled using a 2-L-Limnos sampler (Limnos, Turku, Finland). Undisturbed sediment cores were taken using a Jenkin surface mud sampler (Ohnstadt and Jones, 1982). The cores were sliced and processed in the laboratory within two hours after sampling. To collect water close to the sediment-water interface, a plastic liner with holes drilled at 1-cm intervals was used in a Jenkin surface mud sampler. The holes were closed with plastic tape. In the field, water for dissolved methane was sampled sequentially from prescribed depths above the sediment-water interface (0, 6 and 12 cm above the sediments; 0 cm = water collected at the interface). Standard analysis of water and sediment were performed according to German standards (DEV, 1991).

Gas sampling

Duplicate gas traps (Limnos, Turku, Finland) were located in the NE (1999 and 2000) and SW (only during 2000) basins. The traps consisted of inverted funnels (0.34 m internal diameter) with a flask screwed to the top; these were suspended 0.5 m above the sediment surface regardless of the depth of overlying water. The traps were left *in situ* for one week and then the flasks were closed under water with a butyl stopper and taken. All samples were transported to the laboratory and analysis of the total volume of gas collected and the methane content was commenced within 4 hours.

Methane analysis

Methane was analyzed using a Shimadzu GC-14A gas chromatograph equipped with a 1.5-m carboxen column and fitted with a flame ionization detector. Flow rates were 3.6 l/h for nitrogen as the carrier gas and 3 and 30 l/h for hydrogen and air, respectively, with an oven temperature of 35 °C. Samples of the bubble gas were injected via a gas sample valve fitted with a 0.5 ml sample loop. The loop was flushed with at least 4 times its volume of sample before injection. To determine the concentration of methane dissolved in water, a 40-ml water sample was injected using a glass syringe into an evacuated 140-ml serum bottle that contained a saturated solution of sodium chloride. Under these conditions, methane is degassed quantitatively into the headspace (Heyer and Suckow, 1985; Casper, 1992b). Subsamples (each 500 µl) from the 40-ml headspace were injected directly into the column. Methane concentrations in the sediments were measured from the headspace of stoppered 16-ml vials containing 2 ml wet sediment and 4 ml NaOH (4%). The vials were filled by sub-sampling each layer of sediment with 5-ml plastic syringes, with the luer lock end removed; sediments were cored and displaced into the vials.

To measure methane production rates 4-ml subsamples from each sediment layer were filled into 16-ml vials stoppered with butyl septa. The sediments were degassed, after the addition of 4 ml sterilized lake water, with nitrogen and than incubated for 48 hours at *in-situ* temperature in the dark (Casper, 1992b). Methane production rate was estimated as the increase of methane concentration in the headspace of the sample vials. Methane was analyzed as described above. The concentration measurements were done with duplicate flasks, and methane production in triplicate. From each flask, at least three gas samples were injected. In all cases instrument calibration was performed using standard gas mixtures (1, 10 and 100% CH_4 in N_2; Supelco, USA).

Total bacteria and methanogen counts

Bacteria were detached from sediments by gentle centrifugation (750 × g, 10 min) as described by Furtado and Casper (2000) for sediments with high water content; the efficiency was highest compared to sonication, centrifugation with density gradient or sediment dilutions with tetrasodium-pyrophosphate. The supernatants were fixed with 3.5% formaldehyde (final concentration) and stored at 6 °C. To count the total number of bacteria, samples were diluted with sterilized destilled water and stained with the fluorochrome 4′, 6-diamidino-2-phenylindole, (DAPI, Merck, D; final concentration 5 mg/L) for 10 min. The samples were filtered onto black 0.2 µm polycarbonate filters (Nuclepore Corp.) under low vacuum pressure (Kepner and Pratt, 1994). The filters were embedded in Citifluor AF3 (Citifluor Ltd., UK) after drying. Methanogens were counted using autofluorescence of coenzyme F_{420} (Doddema and Vogels, 1978). Samples were prepared as described with the following exceptions: no DAPI staining; use of white filters and embedding in Citifluor AF1. Counting was performed with a fluorescence microscope (Leica DMBR, HBO 100 W, Filterset D, Germany). From each sample, 10 randomly selected fields or a minimum of 400 cells were counted from each of 3 filters (Schallenberg et al., 1989). Non-parametric Mann-Whitney-tests were performed to test for significance between means at $p < 0.05$.

Fluorescence-in-situ-hybridization

The protocol follows, in general, Manz (1999) with some slight modifications. The oligonucleotide probes (working concentrations 50 ng/µl) were labeled with the fluorochrome Cy3 at the 5′ end (Biometra Göttingen, D). The sequences and the target organisms of the oligonucleotide probes are summarized in Table 2.

The cells of sediment microorganisms were separated also by gentle centrifugation. The supernatants were fixed for 3 hours with 1.75% formaldehyde in 1× phosphate-buffered saline (PBS: 130 mM NaCl, 7 mM Na_2HPO_4, 3 mM $Na_2H_2PO_4$, pH 7.4) and then washed with PBS. The fixed material was diluted in PBS : ethanol (1:1) and stored at –20 °C. Hybridization was done on Teflon-coated glass slides having 8 holes (Marienfeld, D). After air drying, the glass slides with sample material were dehydrated by alcohol (50, 80, 96%).

The warmed hybridization buffer (0.9 M NaCl, 20 nM Tris HCl (pH 7.2), 0.01% sodium dodecyl sulfate (SDS)) contained 30% formamide. The oligonucleotide probes were mixed with hybridization buffer (1 : 9) and added to the cells in each hole of the glass slides. The glass slides were then placed in a wet chamber for 1.5 h at 47 °C for hybridization. After this time, the glass slides were incubated with warmed washing buffer (125 mM NaCl, 20 mM Tris-HCl, 0.01 SDS) for 20 min. Than the glass slides were washed carefully with distilled water. After air drying, materials were embedded in Citifluor AF1. Hybridization was checked under the microscope (filter set N2.1, Leica, D).

Cells were stained by DAPI solution after hybridization. Twenty randomly selected fields or at least 1,000 cells were counted per sample and probe. The amount of oligonucleotide probes hybridized cells were calculated against the total number of cells.

Results

Typical vertical profiles of methane- and oxygen concentrations and of temperature are shown in Figure 2 for the less acidic sub-basin NE and the acidic SW compartment.

Table 2. Sequences and target organisms of used oligonucleotide-probes.

Probe	Sequence (5′– 3′)	Reference	Target group
nonEUB	CGACGGAGGGCATCCTCA	a)	No organism
EUB 338	GCTGCCTCCCGTAGGAGT	b)	Eubacteria
Arch 915	GTGCTCCCCCGCCAATTCCT	b)	Archaea
Eury 498	CTTGCCCRGCCCTT	c)	Euryarchaeota
MB 1174	TACCGTCGTCCACTCCTTCCTC	d)	Methanobacteriaceae
MB 310	CTT GTC TCA GGT TCC ATC TCC G	d)	Methanobacteriaceae
MC 1109	GCAACATAGGGCACGGGTCT	d)	Methanococcaceae
MG 1200	CGGATAATTCGGGGCATGCTG	d)	Methanomicrobiaceae Methanocorpusculaceae Methenaoplanaceae
MSMX 860	GGCTCGCTTCACGGCTTCCCT	d)	Methanosarcinaceae
MS 1414	CTCACCCATACCTCACTCGGG	d)	*Methanosarcina sp.* *Methanococcoides sp.* *Methanolobus sp.* *Methanohalophilus sp.*
MX 825	TCGCACCGTGGCCGACACCTAGC	d)	*Methanosaeta sp.*
MS 821	CGCCATGCCTGACACCTAGCGAGC	d)	*Methanosarcina sp.*

a) Wallner et al., 1993; b) Harmsen et al., 1996; c) Burggraf et al., 1994; d) Raskin et al., 1994.

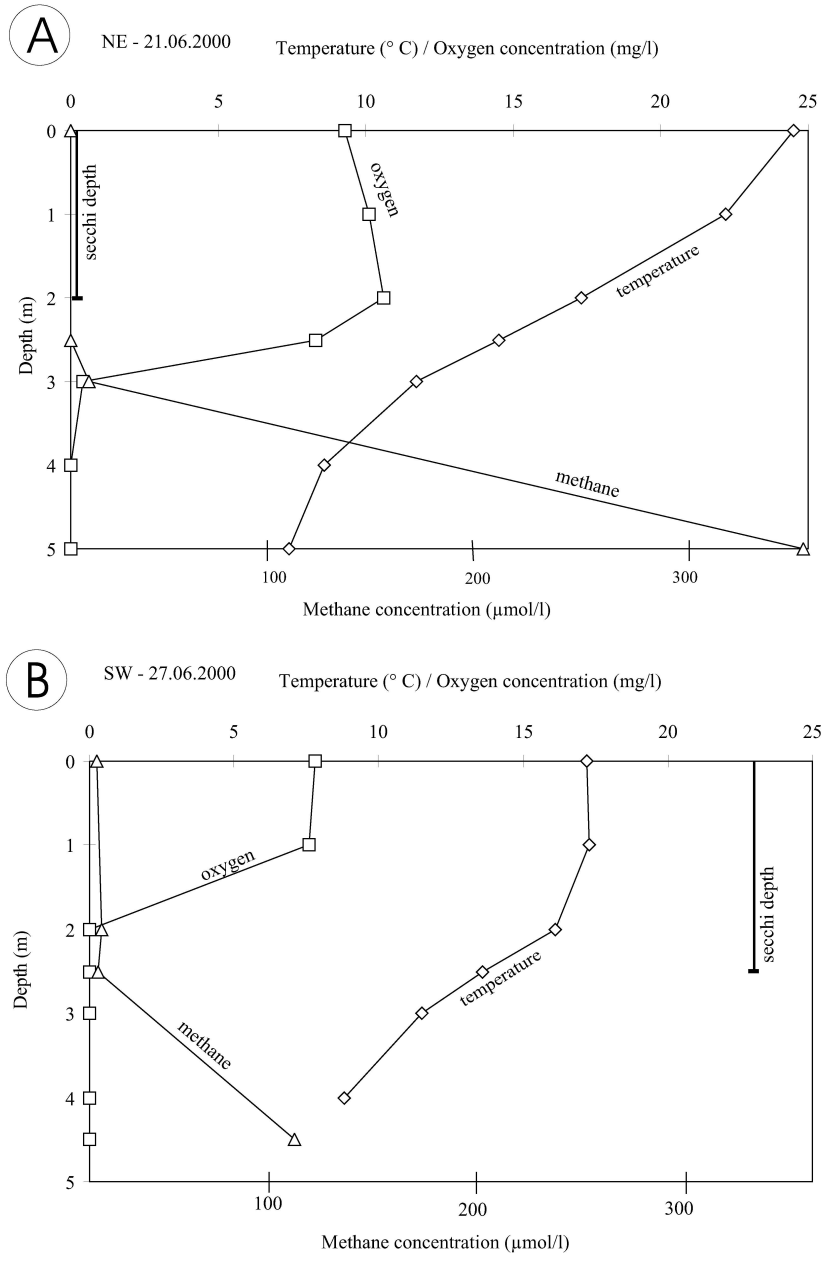

Figure 2. Depth profiles of temperature [°C], methane [µmol/L] – and oxygen [mg/L] concentrations and secchi depths in the water columns of NE- and SW basins of Lake Grosse Fuchskuhle in June 2000 (A = NE – 21.06.2000; B = SW – 27.06.2000).

At the sampling dates in June 2000, both basins were stratified with an anoxic hypolimnion occurring below 3 m (NE) and 2 m (SW). Methane accumulated in the anoxic water layers (Figs. 2, 3). Methane concentrations near the sediment were about three times higher in the NE basin with higher concentrations observed even within the first 15 cm of sediment (Fig. 3).

In the SW basin, no methane production was found in the surface layer and below 15 cm depth, the highest rate was in the 5–10 cm layer (4.6 ± 0.2 µmol L^{-1} d^{-1}). In contrast, methane production was observed throughout the NE basin sediment, except the 10–15 cm layer.

During spring to fall 2000, methane ebullition was detected only in the NE basin (Table 3), beginning in late summer (31.07.) and ending in fall (16.11.). The rates of gas release (77 ml m^{-2} d^{-1}) and also the amount of methane (54.7%) in the gas were found to be very similar between 1999 and 2000 in the NE basin.

The sediment composition was comparable between both basins (Table 4). The top 20 cm of sediments was characterized by high water content (>96%), high amounts of organic material (loss on ignition (LOI) approx. 85% of dry matter (dm)) and low concentrations of calcite. At sampling dates in June 2000, sulfate was

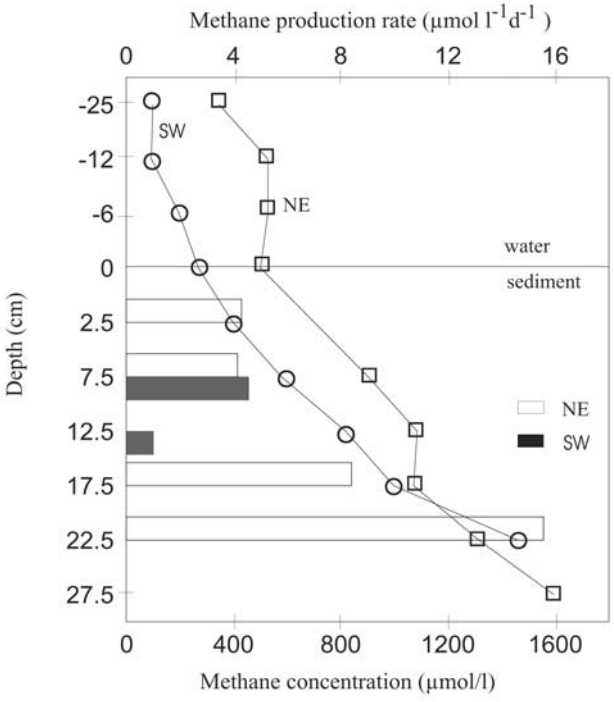

Figure 3. Methane concentration [µmol/L] profiles (given as lines) in the sediment and in the overlying water, and methane production rates [µmol l^{-1} d^{-1}] in NE (white bars) and SW (black bars) basins of Lake Grosse Fuchskuhle (sampling dates as in Fig. 2) Twelve, 6 and 0 cm above the sediment were sampled from Jenkin-sediment cores, the 25 cm sample was for water samples taken with a water sampler near the sediment surface.

detectable only in samples near the sediment surface and pH values were approx. 6.0 throughout the sediment depths studied in both sub-basins.

The total number of bacteria cells revealed from DAPI staining was between $0.95 \pm 0.18 \times 10^7$ and $1.73 \pm 0.31 \times 10^7$ cells/ml wet sediment (Fig. 4). In the NE basin, the highest number of cells (1.73×10^7 cells/ml) were found in the top 0–5 cm. This number was significantly higher ($p < 0.05$) than in the surface layer of the more acidic SW basin ($1.46 \pm 0.35 \times 10^7$ cells/ml wet sediment). In both basins, cell numbers were significantly ($p < 0.05$) higher at sediment surface layers than at greater sediment depth of 16–25 cm (NE $1.1 \pm 0.23 \times 10^7$; SW $1.1 \pm 0.15 \times 10^7$ cells/ml). In contrast, the highest number of cells in the SW basin was found in the 6–10 cm layer ($1.72 \pm 0.27 \times 10^7$ cells/ml; Fig. 4).

The percentage of autofluorescent methanogens within the total DAPI-stained cells increased from 10.1% to 22.3% with sediment depth in the NE basin. This increase corresponded to a cell count increasing significantly ($p < 0.05$) from $1.75 \pm 0.25 \times 10^6$ to $2.57 \pm 0.33 \times 10^6$ cells/ml wet sediment. In the SW basin, the abundance of methanogens was 1.34 ± 0.44 and $2.03 \pm 0.64 \times 10^6$ cells/ml, corresponding to 9.2% to 14.7% of the total bacterial number (Fig. 4).

The fraction of autofluorescent and nonspecifically stained cells as determined with the negativ probe NON338 was low, and was only detected in the upper sediment layer. The signal was mainly derived from the

Table 3. Methane ebullition fluxes and percentage content in the total gas released from the NE (1999 and 2000) and SW (2000) basins of Lake Grosse Fuchskuhle.

		NE		SW 2000
		1999	2000	
Total gas released [a, b]	(ml m^{-2} d^{-1})	76 (0–132)	77 (0–205)	0
Methane released [a, b]	(mmol m^{-2} d^{-1})	1.9 (0–3.6)	2.0 (0–4.2)	0
Methane content [a, c]	(%)	54.4 ± 6.2	55.0 ± 11.3	\
Duration of gas release		23.08.–28.10.	31.07.–16.11.	\
Duration of gas release	days	61	108	0

[a] Average for the release duration based on weekly measurements; [b] Minimum – maximum values in brackets; [c] Standard deviation.

Table 4. Sediment characterization for the NE and SW basins of Lake Grosse Fuchskuhle in June 2000 (NE/SW).

Depth (cm)	pH	SO_4^{2-} (mg/L)	dm (% ww)	LOI (% dm)	$CaCO_3$ (% dm)	Minerals (% dm)
appr. 5 cm a.s.	6.1 / 5.9	2.9 ± 0.3 / 3.3 ± 0.2	\ / \	\ / \	\ / \	\ / \
0–5	6.0 / 5.9	n.d. / n.d.	2.6 / 2.8	84.9 / 83.9	1.8 / 1.2	13.4 / 14.9
6–10	6.0 / 5.9	n.d. / n.d.	3.1 / 3.0	84.7 / 83.6	1.8 / 1.7	13.5 / 14.7
11–15	6.0 / 6.0	n.d. / n.d.	3.6 / 3.1	84.5 / 84.4	1.6 / 0.8	13.9 / 14.8
16–20	5.9 / 6.0	n.d. / n.d.	3.6 /3.6	84.9 / 83.9	1.4 / 2.0	13.7 / 14.2

dm = dry matter, ww = wet weight, LOI = loss on ignition, n.d. = below detection limit, a.s. = above sediment.

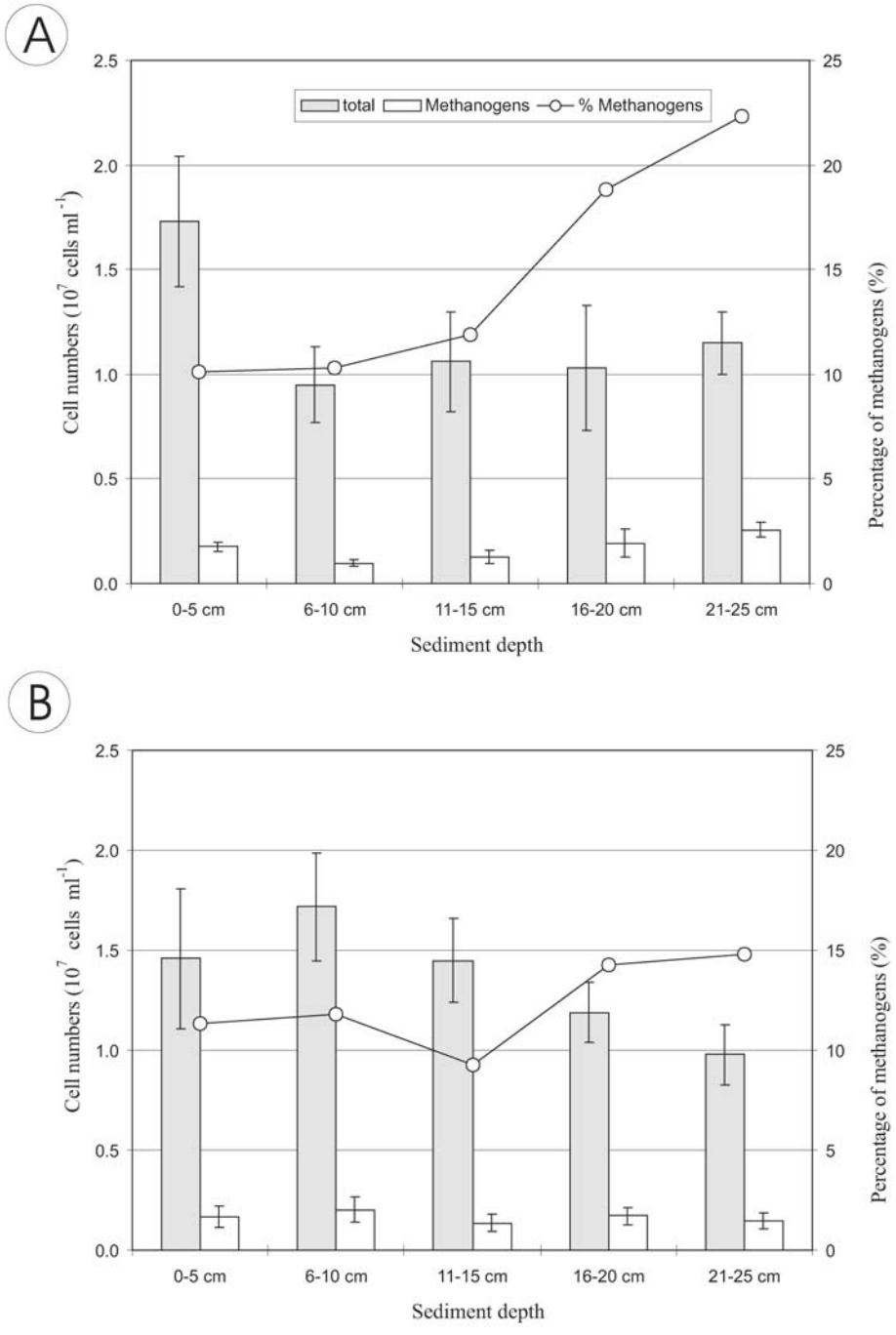

Figure 4. Total bacterial (gray bars) and methanogen (white bars) cell numbers in different sediment layers in NE (A) and SW (B) basins of Lake Grosse Fuchskuhle. The lines indicate the percentage of methanogens as compared to total bacterial cell numbers (standard deviations are given, sampling dates as in Fig. 2).

chlorophyll-containing algae, which could be easily distinguished from the red auto-fluorescent under UV excitation. The abundance of eubacteria cells was higher in the NE than in the SW basin at the sediment surface (Fig. 5). The percentage of EUB-hybridized bacteria was in the range of 9.9 to 21.7% of the DAPI-stained cells. In the NE basin, the portion of EUB-hybridized cells was 21.7% in the surface 0–5 cm sediment. It was markedly higher than 15.7% at the deeper 21–25 cm layer. In contrast, the fraction of EUB-hybridized cells was 15.3% in the SW basin, which was similar to that at the same depth in the NE basin. The fraction of the domain Archaea, reflected from ARC-hybridized cells, varied from 0 to 2.9% of the DAPI-stained cells. Higher abundances of

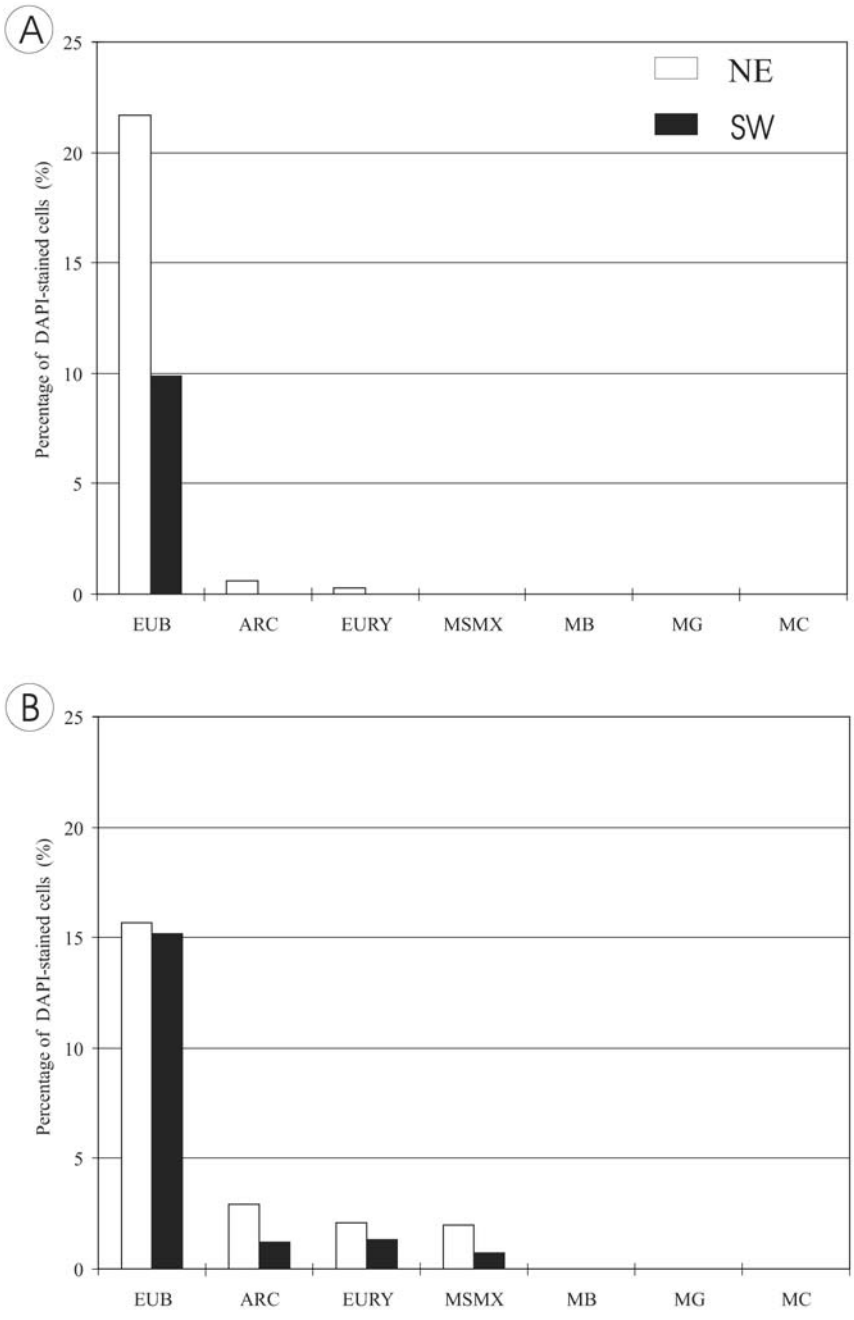

Figure 5. Microbial community structure in the surface (0–5 cm) sediment layer (A) and in a deeper (21–25 cm) layer (B) in NE (white bars) and SW (black bars) basins of Lake Grosse Fuchskuhle. (Sampling dates as in Fig. 2; abbreviations of oligonucleotide probes as in Table 2).

Archaea were detected in the deeper sediment layers of both basins. In the NE basin, the percentages were 0.6% in the surface layer and 2.9% at 21–25 cm depth. No signal was detected from hybridization with probe ARC915 for more than 1,000 DAPI-stained cells from the top 0–5 cm layer, while 1.2% were found in the deeper layers. The EURY-hybridized cell count was very similar to the ARC-pattern.

From all tested methanogens-targeting oligonucleotide probes, only *Methanosarcinaceae*-targeting probe MSMX 860 gave signals for the deeper (21–25 cm) sediment layer. The abundance was 2.0% and 0.7% in the NE and SW basins, respectively. The cell count was nearly the same as the EURY-hybridized cells. In all samples, encountered hybridized cells were less than 10% of the autofluorescent cells (Figs. 4 and 5).

Discussion

From studies in enclosures or experimentally divided lakes, it is known that basic limnological parameters such as water chemistry and community structure can change markedly (Lund and Reynolds, 1982). Because Lake Grosse Fuchskuhle was chosen as an experimental lake for food-web experiments (Kasprzak et al., 1988), the first studies after division dealt with evaluating changes in community structure and water chemistry in the different basins (e.g., Kasprzak, 1993; Koschel et al., 1995; Hehmann and Krienitz, 1996; Ronneberger and Anwand 2000; and Hehmann et al., 2001). To summarize, the basic limnological characteristics and annual dynamics were similar to those of the original lake from 1986 until 1992. The original lake and the two basins formed from the first division were sporadically stratified and the water column was oxic for practically the entire year (Babenzien et al., 1991). After the second division, the four basins developed differently with similarities between the eastern as well as between the western compartments. Both eastern basin compartments became less acidic (pH ~ 6–7). On the other hand, the western basin compartments continued to receive humic substances from the adjacent peat which kept the pH low (ca. 4–5) and the DOC high (10–30 mg/L) in the water column (Sachse et al., 2001). It seems that the pronounced differences between the basins were mainly related to the changes in morphometry and the influence of peat in the catchment and not to the later gradual stocking of perch (Šimek et al., 1998; Koschel et al., 1995; and Ronneberger and Anwand, 2000).

In June 1989, three years after division into two basins, oxygen saturation levels of >90% near the sediment surface were observed (Casper, 1992b). Because of sufficient oxygen supply to the hypolimnia, the oxic/anoxic interface, where methane was oxidized, was close to the sediment surface and this gas did not accumulate in the deep water layers. Methane concentrations reached only 0.3–10 µmol/L in the water column near the sediment surface during this earlier period (Casper, 1992a, b).

In the present study, about 10 years after final division, the water columns remain strongly stratified during the entire summer stagnation period until October when anoxic deep water layers develop. The methane concentrations near the sediment surface are now 10 times (SW c. 100 µmol/L) and 35 times (NE c. 350 µmol/L) higher than earlier. Such concentrations are typical for stratified lakes, where the oxic/anoxic interface has moved upwards from the sediment surface into the overlying water column, and other reduced gases such as H_2S become noticeable. Bittl and Babenzien (1996) described the accumulation of sulfides as high as 5 mg/L in the hypolimnia in Lake Grosse Fuchskuhle.

The spatial and temporal patterns of redox-sensitive processes in sediments, like methanogenesis, were strongly influenced by the oxygen concentration in the overlying water (Casper, 1992a). In eutrophic lakes, methanogenic activity can be found at the sediment surface, in the same horizon as sulfate reduction (Casper, 1996), when anoxic conditions are present. Although in both basins the sediment surface was anoxic only in the less acidic NE basin, methanogenesis was already measured in the top sediment layer. During the stagnation period, the methanogenic activity in the NE basin increased and led to the release of gases, mainly of methane to the water and then the atmosphere. An oversaturation of the relatively insoluble methane (saturation in freshwater is about 1.6 mmol/L at 20 °C, Yamamoto et al., 1976), followed by the formation of so called "gas bags" in the sediment is the prerequisite for ebullition losses to the atmosphere (Casper et al., 2000b). In the NE basin, ebullition occurred in the late summer at a rate of about 2 mmol m^{-2} d^{-1}. These values are in the range for freshwater lakes as summarized by Smith and Lewis (1992) (2.6 ± 0.5 mmol m^{-2} d^{-1}). In the SW basin, no gas release could be measured in 2000, indicating lower methanogenic activity and gas concentrations in the sediments; this was the situation for the entire year.

The distribution pattern of bacteria and methanogenic archaea did not reflect the sediment methanogenesis activity. In both basins, methanogens were present in the surface 25 cm of sediments but methanogenic activity could only be measured in some of the layers. In the top sediment layer (0–5 cm), both the total bacterial and the methanogen cell numbers were higher in the less acidic NE basin. The biomass of different trophic levels (Hehmann and Krienitz, 1996; Šimek et al., 1998; and Koschel et al., 1995), and indirectly the sedimentation rate of labile organic carbon compounds, differed between the two basins, but the differences did not explain the failure of methanogenic activity in the top sediment layers of the SW basin. Because the main parameters influencing methanogenesis – temperature, pH, redox potential, other electron acceptors – were more or less comparable between both basins, it seemed that the composition of DOC with higher amounts of peat-borne humic substances in the SW basin (Sachse et al., 2001) inhibit the methanogens or syntrophic microorganisms that degrade polymeric substances into useable substrates for methanogens. As shown by Sachse et al. (2001), the concentration of humic acid substances differed only slightly between the basins, but the quality of the substances differed markedly. The inflow through the peat led to a higher amount of humic substances in the SW basin, while in the NE basin the amount of LMWA (low molecular weight acids) was much higher. The availability of labile organic material could also enhance the rate of methanogenesis in the sediments. Influences of humic

substances on enzymatic activities, e. g. on alkaline phosphatase, were suggested by Stewart and Wetzel (1982). In addition, methanogenesis was shown to be inhibited by the addition of a humic model compound, anthraquinone-2,6-disulfonate (AQDS, Cervantes et al., 2000). The AQDS reduction outcompeted the methanogenesis with the AQDS acting as terminal electron acceptor.

In the NE basin, the fraction of methanogens as total cell numbers peaked in the 21–25 cm-depth layer, where the highest rates of methanogenesis were also measured.

Although the detection rates were relatively low when using fluorescence *in situ* hybridization for community structure studies in the sediments of this lake, it is likely that only acetate using methanogens belonging to the *Methanosarcinaceae* were detectable. Conrad (1999) reviewed the contribution of hydrogen to methane production in different methanogenic environments and described an expected rate of 33% for utilization of H_2. He distinguished between low (0–10%), normal (15–45%) and high (70–100%) hydrogen contribution rates. According to Schulz and Conrad (1996), Lake Constance was the only environment where acetate users were the only observed methanogens. Nercessian et al. (1999) analysed the community structure of methanogens in a peat bog using a DNA sequencing approach. They found clones of hitherto uncharacterized sequences closest related to *Methanosarcina barkeri*, also an acetate user. In Lake Rotsee sediments, Zepp Falz et al. (1999) observed a dominance of acetoclastic methanogenesis with a predominance of *Methanosaeta* spp. These groups represented about 90% of the archaeal population. A combination of oligonucleotide probing and PCR amplification with methanogen primers, used in sediment cores of a peat bog, showed that the *Methanosarcinaceae* targeting oligonucleotide probe MSMX 860 were most widespread and were found in all depths below 8 cm (McDonald et al., 1999). In deeper layers of this peat bog, *Methanobacteriaceae* were also detected. Phelps and Zeikus (1984) explained the high contribution (96%) of acetate users in the mildly acidic Knaack Lake by a larger contribution of homoacetogenesis. Sulfate reducing or homoacetogenic bacteria can use hydrogen and lower the H_2 threshold so that hydrogen utilization by methanogens is not possible (Lovley, 1985; Conrad, 1999). In Lake Grosse Fuchskuhle, sulfate reducing activity was found in sediment layers above the methanogenic layers (Babenzien et al., 1991).

Summary

The study basins of this artificially divided lake were different in their methanogenic activities. The NE-basin, characterized by higher pH-values in the water column, was found to have a higher methanogenic activity and more methane accumulated in the hypolimnium. Methanogenesis was detected in the NE-basin at the sediment surface and in deeper layers, and led to the ebullition-release of nearly 2 mmol methane m^{-2} d^{-1} to the atmosphere. The abundance of methanogens did not reflect the different activity patterns in the compartments. In both basins, only methanogens related to the family of acetate – using *Methanosarcinaceae* were found in the sediments. The division of the lake mainly impacted the surface and/or near surface sediments. The differences between the studied sub-basins seemed to result from the quality of the organic matter, namely with humic acids as main components in the peat-related acidic SW subbasin. Further studies, especially concerning the role of humic substances in the basins and their inhibitory effects on sediment microbes, are needed.

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