

A modified diffusion-based methane sensor and its application in freshwater sediment

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Abstract

Methane is the main end product of anaerobic degradation in freshwater sediments. The flux of methane from the sediment into the water column, and possible methane oxidation at the sediment surface, can be estimated on the basis of its distribution in the sediment and the overlying water. Nonetheless, the quantification of methane in sediments at high spatial resolution is difficult, owing to its chemical inertia. In this study, we describe the improvement of a diffusion-based methane sensor and its application in freshwater sediment cores. The sensor consists of a steel cannula with small openings, which are covered by thin silicone tubing. Methane diffuses into the cannula and is flushed directly to a flame ionization detector for quantification. With this sensor, methane profiles in freshwater sediment can be measured at high spatial (1.5 mm) and temporal (3 minutes per measurement) resolution. The detection limit was $2 \mu\text{mol L}^{-1}$. The relative accuracy of the sensor was $\pm 15\%$ with a precision of $\pm 7.5\%$. Analysis of fine-scale methane profiles in littoral and profundal sediments of Lake Constance indicate that owing to structural inhomogeneities and temporal changes a high-resolution analysis is required to allow exact calculations of diffusive fluxes and turnover rates of methane.

Introduction

In anoxic freshwater sediments, methane production is the dominant process in the anaerobic degradation of organic matter. In littoral sediments with a high organic input and seasonally high temperatures, methane often accumulates to supersaturation (Thebrath et al. 1993). Only a minor part of this methane reaches the atmosphere because aerobic and anaerobic bacteria can reoxidize methane within the sediment or in the overlying water (e.g., Liikanen et al. 2002). Because methane is an important greenhouse gas (Lelieveld et al. 1998), an exact quantification of methane fluxes from sediment into the atmosphere is essential for a better understanding of the impact of methane on the global budget of greenhouse gases. Reliable calculations of methane production and consumption rates and of methane fluxes require the analysis of methane concentration profiles in sediment at high spatial resolution. So far this resolution is unsatisfactory.

The conventional method used to determine methane concentrations in the sediment is the headspace method (Kiene and Capone 1985): sediment cores are sliced, and the slices are extracted in closed vials in which the methane concentration

is determined by gas chromatography. These measurements are time-consuming, the spatial resolution is > 1 cm, and the sample material has to be destroyed. Additionally, part of the methane is lost in the slicing process. In the top 5 cm, methane profiles measured with the headspace method show a poor resolution; thus, for flux calculations the gradient between the concentration of methane in the water and in the surface sediment at 1- to 2-cm depth has to be extrapolated (Huttunen et al. 2006; Roy et al. 1996). This extrapolation is rather inaccurate, however, because for most sediment parameters steep gradients occur just in the top few millimeters (Gieseke et al. 2005).

A further development in methane determination was the use of a microbiosensor, e.g. Damgaard et al. (2001). A culture of methane-oxidizing bacteria is immobilized within an oxygen sensor. Oxygen consumption by the bacteria is measured and converted to the methane concentration in the environment. This system works only in the absence of exogenous oxygen, and it is difficult to maintain its functionality over extended periods of time. Therefore, this system has not been commonly applied.

Another development are diffusion-based micro-sensors where methane diffuses through a membrane into a cannula. The collected methane is flushed to separate vials, and these are further analyzed by the headspace method (Rothfuss and Conrad 1994), or is flushed to a photoacoustic detector (Rothfuss et al.

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1996) or a mass spectrometer (Lloyd et al. 1996). The use of the headspace method is again time-consuming, and the sensitivity and reproducibility are low because subsamples have to be taken with a syringe. Furthermore, photoacoustic detectors and mass spectrometry are expensive and require a lot of technological expertise, thus limiting their application.

Methane in the water column is measured by the same principle. Methane diffuses through a membrane to achieve phase separation between the liquid phase and the gas detection space. In most cases, the detector is a semiconductor that changes its resistance in the presence of methane (or hydrocarbons) (Christodoulou et al. 2003; Esser 2002). However, the diameter of the membrane in such methane probes is approximately 4 cm, which makes them far too large for use in sediments. Alternatively, optodes can be applied to measure oxygen, nitrate, pH, and other parameters (Huber et al. 2000; Hulth et al. 2002; Tengberg et al. 2006), but not methane because of its high stability. Other techniques such as photoacoustic laser detectors, optical sensors, or semiconductors (Grossel et al. 2006; Massie et al. 2006; Yatsimirskii et al. 2005) can be applied only for methane measurements in the atmosphere.

In the present study, we present a further development of the methane sensor described by Rothfuss et al. (1994). The system was improved by connecting it directly to the detector of a gas chromatograph, standard laboratory equipment. We applied this improved methane measurement technique to freshwater sediments. The new instrument provides an easy way to obtain more detailed information on the fine scale distribution of methane in surface sediments.

Materials and procedures

Materials and setup—The sensor consists of an inner and an outer cannula (Figure 1). The outer cannula is 115 mm long and has an outer diameter of 1.07 mm (stainless steel cannula; Hamilton, N719, gauge 19, side-port needle). The inner cannula is 112 mm long (stainless steel cannula; Hamilton, N727, gauge 27, straight opening). The total inner volume of the sensor is 30 μL , as calculated from the dimensions of the cannula. A thread to screw the cannula into the housing of a Beckman HPLC autosampler replaced the original Luer opening of the cannula. Additional holes were drilled close to the tip of the outer cannula, either 2 holes of 0.7 mm ($A = 0.77 \text{ mm}^2$) or 4 holes of 0.5 mm diameter ($A = 0.79 \text{ mm}^2$).

Once filled with a silicone membrane, these holes allow the diffusion of methane into the sensor. In the original work the silicone membrane was a mixture of 80 μL siloprene K1000, 10 μL cross-linking reagent K11 (Fluka, Steinheim, Germany), and 10 μL dichloromethane (P. Frenzel, personal communication). However, this membrane was delicate to apply and not very resistant to fine-grained sediment. Thus, we replaced the initial silicone membrane with thin silicone tubing ($\varnothing_o = 0.5 \text{ mm}$, $\varnothing_i = 0.3 \text{ mm}$; Reichelt Chemietechnik, Heidelberg, Germany), giving a wall thickness of 100 μm .

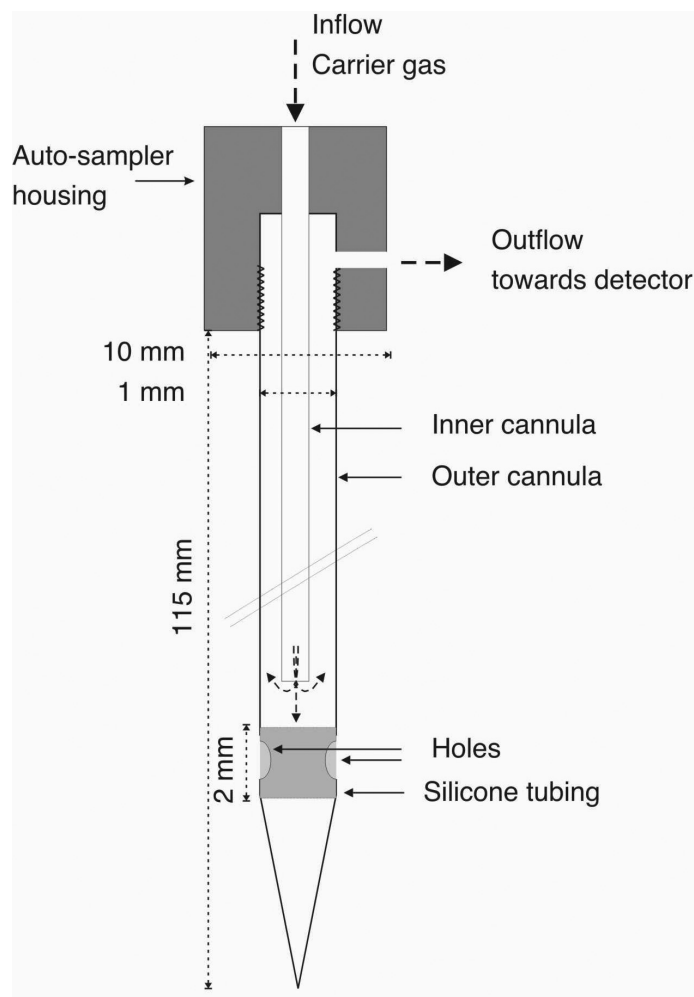


Fig. 1. Sketch of the methane sensor consisting of an inner and an outer cannula. Holes at the sensor tip are covered with a silicone membrane. The carrier gas enters the sensor via the inner cannula, flushes the volume of the outer cannula, and leaves for the detector at the top.

The sensor was connected via 1/16-inch Teflon tubing (Alltech, Germany) to the valves and the detector (Figure 2). The distance between the valves and the sensor was approximately 0.5 m. To reduce the pressure in the cannula, no separation column was used, and the cannula was connected directly to the flame ionization detector of a Mikrolab Aarhus gas chromatograph. The temperatures of the oven and detector were 140 $^{\circ}\text{C}$ and 160 $^{\circ}\text{C}$, respectively. The gas stream was 24 mL min^{-1} N_2 as the carrier gas, 23 mL min^{-1} H_2 , and 72 mL min^{-1} synthetic air. As the pressure of the carrier gas also acted on the silicone membrane of the sensor, the pressure was kept as low as possible, 0.5 bar at the N_2 cylinder.

The baseline of the integrator reacted sharply to pressure changes between the 2 positions of valve 1, i.e., the baseline dropped and recovered at a higher level. However, integration of the peaks via peak height resulted in reproducible values (chromato integrator; Merck-Hitachi D-2000). The methane

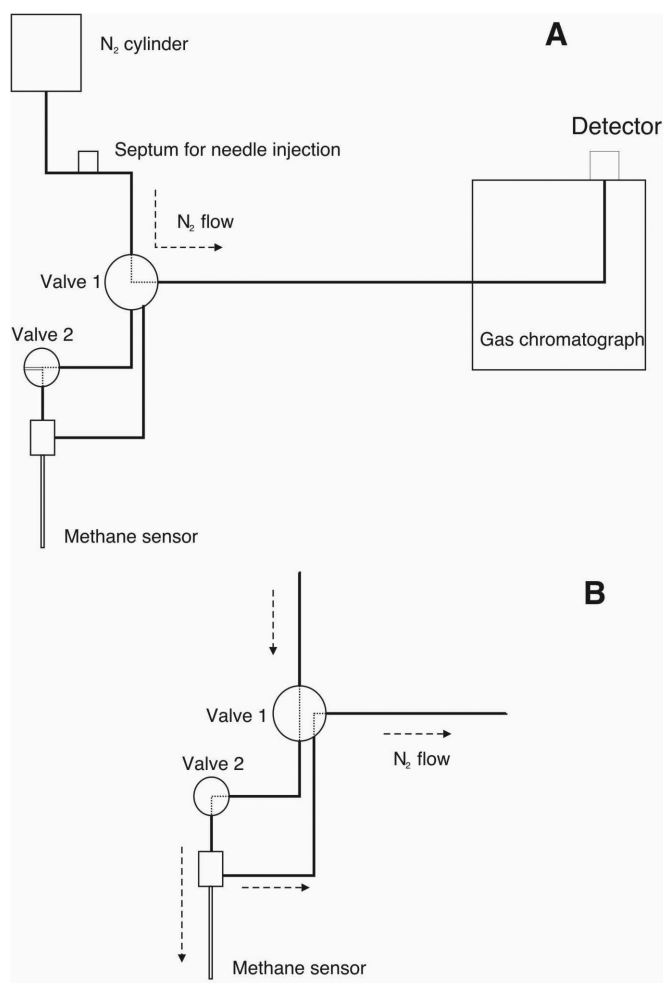


Fig. 2. Flow chart for the carrier gas through the methane sensor to the detector. (A) Valve 1 disconnects the sensor from the gas flow and methane diffuses into the sensor. Valve 2 releases pressure from the sensor prior to the diffusion time. (B) Valve 1 connects the sensor with the gas flow, the sensor is flushed, and the methane is transported to the detector.

peak appeared after 30 s, and no other peaks were ever detected, not even when measuring within the sediment.

Every measurement cycle started with a diffusion period to allow methane to diffuse into the cannula, while valve 1 (Valco 4-port valve; Alltech) was in position A and N₂ was flowing to the detector (Figure 2A). After 3 min diffusion time, valve 1 was turned to position B, and N₂ flushed the methane from the sensor directly to the detector (Figure 2B). For the next 100 s, the cannula was flushed, and the methane peak was integrated. Then valve 1 was turned back to position A, valve 2 (Hoke 3-port valve) was opened for 15 s to release pressure from the system, and the next measurement cycle started. Additionally, samples for measuring gas standards could be injected into a separate septum via a syringe (Figure 2A).

Calibration of the sensor—Before each measurement, the sensor was calibrated with standard solutions of methane in

water. The standard solutions were prepared as follows: glass bottles (100 mL) were filled with 70 mL water, and the exact volumes of the gas and fluid phases were determined by differential weighing. Bottles were closed with butyl stoppers, and various amounts of pure methane were added through the stopper to produce different methane concentrations in the water. After equilibration, the gas phase was analyzed for methane, and the corresponding methane concentration in the water was calculated according to the formula of Flett et al. (1976). Alternatively, after sensor calibration the methane concentration of the water was determined by analyzing 1 mL water subsamples 3 times with the headspace method. At the bottom of the bottle, a glass port closed with tubing allowed insertion of the cannula into the fluid phase with minimal loss of water and gas exchange. Each bottle was sampled in triplicate and shaken thoroughly between the measurements. The peak heights as measured by the sensor were calibrated using the calculated methane concentrations of the fluid phase. Freshly distilled, methane-free water was included in the calibration with 0 μ M methane.

Because the methane sensor described is based on diffusion, the porosity of the environment is of critical importance. Therefore different “environments” were used for calibration: water, 0.2% agarose, a mixture of glass beads, and a sediment slurry. The mixture of glass beads consisted of 80 g glass beads (\varnothing 0.45 mm) and 10 g quartz sand in 70 mL water. For the sediment slurry, sediment from the study site was air-dried and homogenized, and 50 g was then mixed with 70 mL water.

The sensor was tested at room temperature. Calibrations and measurements in the sediment were performed at in situ temperatures (4 to 20 °C) in a cold water bath. Standards and samples were handled at the same temperatures.

Sediment measurements—Experiments were carried out with littoral and profundal sediments from Lake Constance, Germany. The littoral sediment consisted of fine sand with a porosity of 0.62 from which cores (4.4 cm \varnothing , 20 cm length) were taken by scuba diving at 2 to 4 m water depth. The profundal sediment consisted of fine-grained material and clay with a density of about 1.1 g mL⁻¹ and a porosity of 0.85. Cores of profundal sediment (\varnothing 8 cm, length 30 cm) were taken in August 2004 with a ship-borne multicorer at 80 m water depth. Within 0.5 to 3 hours, the littoral and profundal sediment cores were brought to the laboratory where, to simulate in situ conditions, the cores were surface-flushed continuously with aerated lake water at in situ temperature. The sensor had been calibrated before, and measurements started immediately upon arrival. Approximately 3 h later, cores could be used for conventional extraction.

The sensor was moved inside the sediment by a micromanipulator (Mikromanipulator MM33; Märzhäuser, Wetzlar, Germany). A dissecting microscope was used to check when the membrane-covered tip entered the sediment surface (0 mm depth). During measurements, the sediment cores were kept at their respective in situ temperatures.

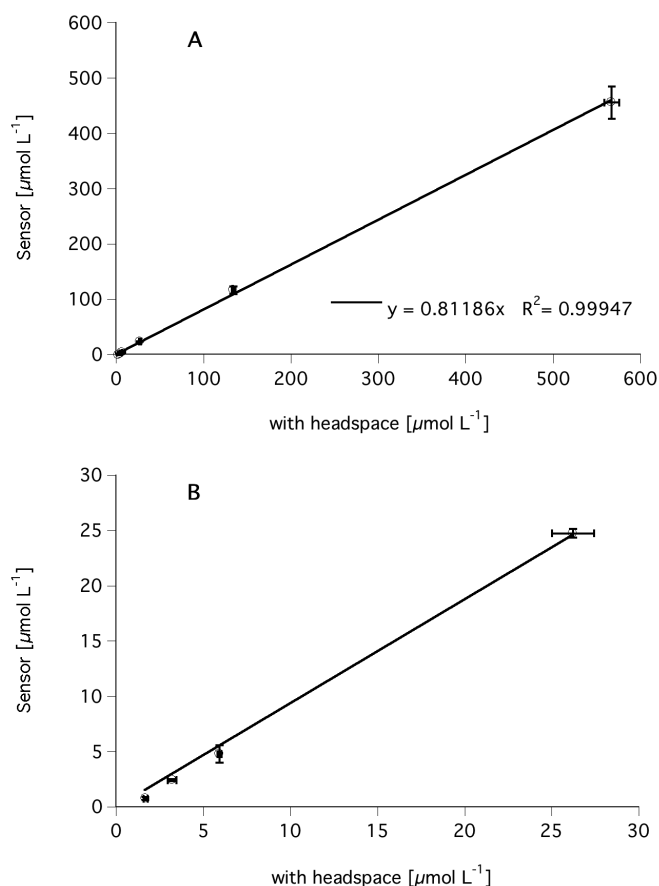


Fig. 3. Methane concentrations determined with the headspace method versus determination with the sensor in the same vessel. Shown is the mean of 3 determinations, each with the standard deviation. A shows the whole concentration range, while B is a close-up for the lower concentration range.

Methane concentrations obtained with the sensor were compared with those obtained after conventional extraction. For this the sediment cores were subsampled with a cutoff syringe at 1-cm intervals from the surface to 3 cm depth. Approximately 1 mL sediment was transferred into a 30-mL glass vial filled with 2 mL of 1 N NaOH. Every sediment core was sampled in triplicate with 3 syringes. The headspace was then analyzed for methane (Kiene and Capone 1985). All samples were stored upside down and analyzed within 2 days.

The diffusive methane flux (J_D) was calculated from the methane concentrations using Fick's first law: $J_D = -D_s \cdot \phi \cdot (dc/dz)$ ($\text{nmol cm}^{-2} \text{h}^{-1}$), with ϕ = porosity of the sediment (0.62 for littoral sediment and 0.85 for profundal sediment), D_s = sediment diffusion coefficient, and dc/dz = the measured methane concentration gradient in the top layers. The bulk sediment diffusion coefficient (D_s) was calculated from the molecular diffusion coefficient (D) by correction for porosity and tortuosity using the empirical equation ($D_s = D \cdot \phi^2$) (Lerman 1979) with $D_s^\circ = 1.13 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and $D_{25}^\circ = 2.00 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Broecker and Peng 1974).

Assessment

Sensor characteristics—The precision of the sensor is defined by the coefficient of variation ($cv = \text{standard deviation} \cdot 100 / \text{mean}$) (Kellner et al. 2004) determined in the calibrations. The cv was low at 7.5 ± 4.6 ($n = 57$). The accuracy of the sensor was determined by measuring the methane concentration in a calibration vessel, first with the sensor and then with the headspace method (Figure 3). The accuracy was calculated from the difference between the sensor and the headspace measurements (Kellner et al. 2004). The absolute accuracy for the lower range (from 1 to 26 $\mu\text{mol CH}_4 \text{ L}^{-1}$, $n = 4$) was $\pm 0.96 \mu\text{mol L}^{-1}$ (Figure 3B). For the whole range of concentrations (from 1 to 566 $\mu\text{mol CH}_4 \text{ L}^{-1}$, $n = 6$, Figure 3A) the relative accuracy was $\pm 15\%$. With aerated distilled water as a blank, no peak was detected. The threshold for reliable detection of peaks was around 1 $\mu\text{mol CH}_4 \text{ L}^{-1}$. Taking the average signal for 1 $\mu\text{mol L}^{-1}$ and adding 3 times its standard deviation, the detection limit was 2 $\mu\text{mol CH}_4 \text{ L}^{-1}$.

A memory effect was never encountered when measuring in sediment with maximal concentrations of around 200 μM methane, but only after calibration with standards containing more than 700 μM . With no memory effect observed in our investigated cores, each new profile started with 0 μM methane in the above lying water. The memory effect could be a problem when measuring sediment with methane concentrations above 500 μM . When measuring at high concentrations, 1 to 2 measurement cycles in water should be sufficient to return the sensor to background levels. The memory effect depended also on the silicone membrane applied. When applying the fluid silicone onto the cannula, some extra silicone probably solidified inside the cannula, acting as a reservoir for methane. When using the thin silicone tubing the memory effect was negligible. In contrast to methane sensors with a closed detector space, in our system methane does not have to diffuse out again, but is flushed out continuously with the carrier gas.

Silicone has a high permeability for many gases and a low selectivity (Stauder 1992). Additionally, the flame ionization detector and the GC were adapted for methane detection. Therefore no peaks other than methane peaks were observed.

A rough estimation of the diffusion range (s) in the water surrounding the tip of the sensor is given by $s = \sqrt{2 \cdot t \cdot D_s}$ (Rothfuss and Conrad 1994), with t = exposure time and D_s = bulk sediment diffusion coefficient. D_s was calculated from the molecular diffusion coefficient (D) by correction for porosity (ϕ) and tortuosity using the empirical equation ($D_s = D \cdot \phi^2$) with $D_{23}^\circ = 1.93 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (Broecker and Peng 1974) and a porosity of 0.62. With a standard exposure time of 3 min and a value for $D_s = 7.42 \cdot 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, the diffusion range is about 0.5 mm. Adding twice this range to the diameter of the membrane (0.7 or 0.5 mm), the spatial resolution of the sensor was 1.7 or 1.5 mm, respectively.

The diameter of the outer cannula and the size of the holes limit the spatial resolution of this sensor. Miniaturization of the cannula would increase the spatial resolution, but would

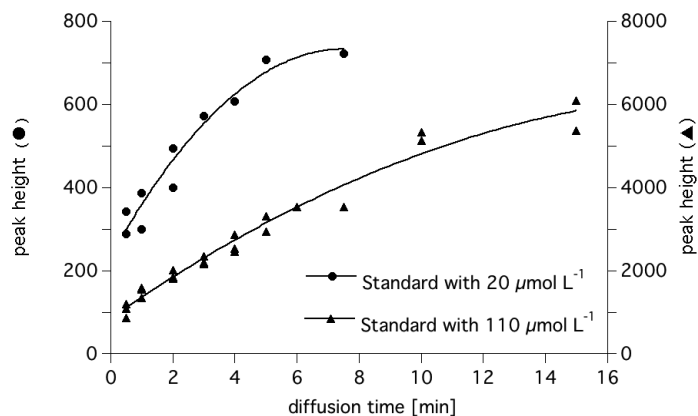


Fig. 4. Signals obtained with the methane sensor exposed for different diffusion times in a methane standard solution. Results of measurements with standard solutions of 20 μM and 110 μM methane are shown.

decrease the sensitivity. Furthermore, in the sediments investigated significant changes in methane concentration appear to occur not in the top few millimeters but at depths > 10 mm. Therefore, it is advantageous if the cannula is sturdy and can be pushed deep into the sediment.

Calibration—Our sensor is based on the diffusion of methane into the cannula. Diffusion is affected by temperature, time, and the physical structure of the surroundings. The effect of temperature is not explicitly addressed in this study, but was circumvented by handling standards and samples at the same temperature. Effects of diffusion time and diffusion medium were investigated in the following experiments.

Because diffusion is a time-dependent process, the signal obtained from the sensor depends on the exposure time, i.e., the time during which methane can diffuse into the cannula. As shown in Figure 4, the longer the diffusion time, the higher the resulting peak. After diffusion times > 6 min, the signal leveled off. With a low-concentration standard (20 μM), no signal could be detected after 0.5 min diffusion time. As a compromise between sensitivity, spatial resolution, and total time needed for one profile measurement, a standard diffusion time of 3 min was chosen for both the standards and the sediment samples. It was reported that in soil when the exposure time was extended to more than 4.5 min, the signal of the sensor was no longer linear (Rothfuss and Conrad 1994). Therefore these authors chose a standard diffusion time of 2 min for working in methane-rich paddy soils (Rothfuss et al. 1994).

Diffusion of methane into the sensor also depends on the porosity of the surrounding medium (Lerman 1979). Therefore calibrations in water were compared with calibrations in a sediment slurry, water with glass beads, and 0.2% agarose. Three calibrations were made for each medium. The calibration curve was steepest in water (Figure 5), whereas the calibration curves obtained with agarose, sediment slurry, and glass beads were less steep, with $83\% \pm 3.7\%$, $73\% \pm 1.2\%$, and $70\% \pm 4.5\%$ of the slope in water, respectively.

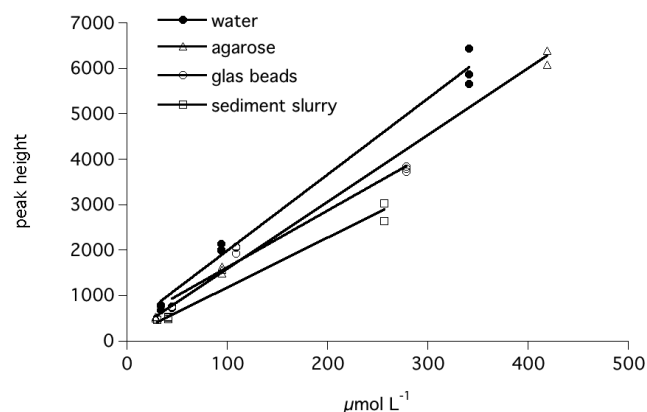


Fig. 5. Calibration curves of the methane sensor in water, agarose, sediment slurry, and glass beads; each incubation condition was tested with 3 sets of measurements at different concentrations.

Thus it is necessary to perform calibrations in media of similar diffusivity as that of the medium being analyzed. For methane measurements in bacterial cultures growing in agarose gradients, calibration was performed with agarose standards (Bussmann et al. 2006). For methane measurements in sediment, we used glass beads to mimic the in situ diffusivity (see “comparison with head space analysis”). However, it has to be taken into account that such a procedure is difficult if the diffusivity changes also within one sediment core.

Comparison with headspace analysis—To validate the new methane sensor, the results of sensor measurements were compared with those from conventional headspace analysis. After calibration, the methane concentration in a sediment core was measured 3 times with the sensor. Afterward the same core was sampled for headspace analysis. No significant difference was found between results of the headspace analysis and sensor measurements, calibrated either with glass beads (Figure 6, paired sign test, $n = 15$, $P = 0.42$) or sediment slurry (data not shown). However, at the 2- to 3-cm depth interval the methane concentration determined by headspace analysis was lower than that obtained with the sensor, probably owing to methane escaping during sampling and filling of the vials for headspace analysis. Because glass beads were easier to handle, they were used for routine calibration. Direct comparison between conventional methane analysis of a sediment core and measurements with the methane sensor revealed that the values obtained did not differ significantly if the sensor was calibrated in the presence of glass beads or sediment slurry. For sediments with other diffusional characteristics, the calibration may need to be readapted.

Comparison of headspace analysis and sensor measurements demonstrated how much information is lost by conventional headspace analysis. Detailed information on the slope of methane increase in the top 10 mm is essential for calculations of methane flux or oxidation rates.

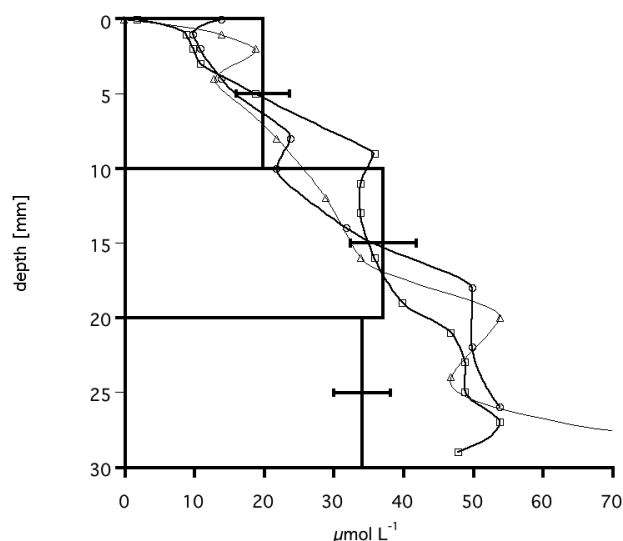


Fig. 6. Methane profile in a littoral sediment core measured with conventional headspace analysis (bars with standard deviation of 3 samples) or measured with the methane sensor (3 measurement series) calibrated with glass beads.

Sediment measurement—Methane profiles in littoral sediment taken at the end of winter (February 2005) were compared with profiles in littoral and profundal sediment taken in summer (August 2004) (Figure 7). In February, almost no methane was detected in the upper 25 mm ($1 \pm 2 \mu\text{mol L}^{-1}$, $n = 26$, Figure 7A). Below this layer, maximal methane concentrations of $50 \mu\text{mol L}^{-1}$ were reached in one profile at 31 mm depth, whereas in 2 other profiles methane concentrations increased less steeply, and maximum values of 37 and $18 \mu\text{mol L}^{-1}$ were reached at 50 mm depth. In August, low methane concentrations were found only in the top 16 mm with concentrations somewhat higher than in February ($4 \pm 2 \mu\text{mol L}^{-1}$, $n = 32$, Figure 7B). However, below 20 mm depth, methane concentrations increased sharply to a maximum $140 \mu\text{mol L}^{-1}$ at 50 mm depth. In profundal sediment, methane concentrations in the upper 10 mm were higher than in the littoral sediment ($10 \pm 8 \mu\text{mol L}^{-1}$, $n = 18$, Figure 7C) but also exhibited greater variability. Below 10 mm sediment depth, methane concentrations increased sharply to much higher values than in the littoral zone, $236 \mu\text{mol L}^{-1}$ at 50 mm and almost $400 \mu\text{mol L}^{-1}$ at 70 mm (these values are out of the range shown in the figures).

Assessment of the variability of methane distribution within neighboring areas is important to estimate how many parallel measurements are necessary to obtain realistic values for one sediment core. We therefore calculated the number of profiles needed to assess a given natural gradient with sufficient accuracy. In one sediment core (littoral, 12 July 2005) we repeated the measurements 7 times in the upper 30 mm and 3 times at the depth from 30 mm to 60 mm (Figure 8). All measurements were 5 mm apart from each other. Methane concentrations in this core were overall quite low. Down to 14 mm depth, methane concentrations were mostly below the detection limit

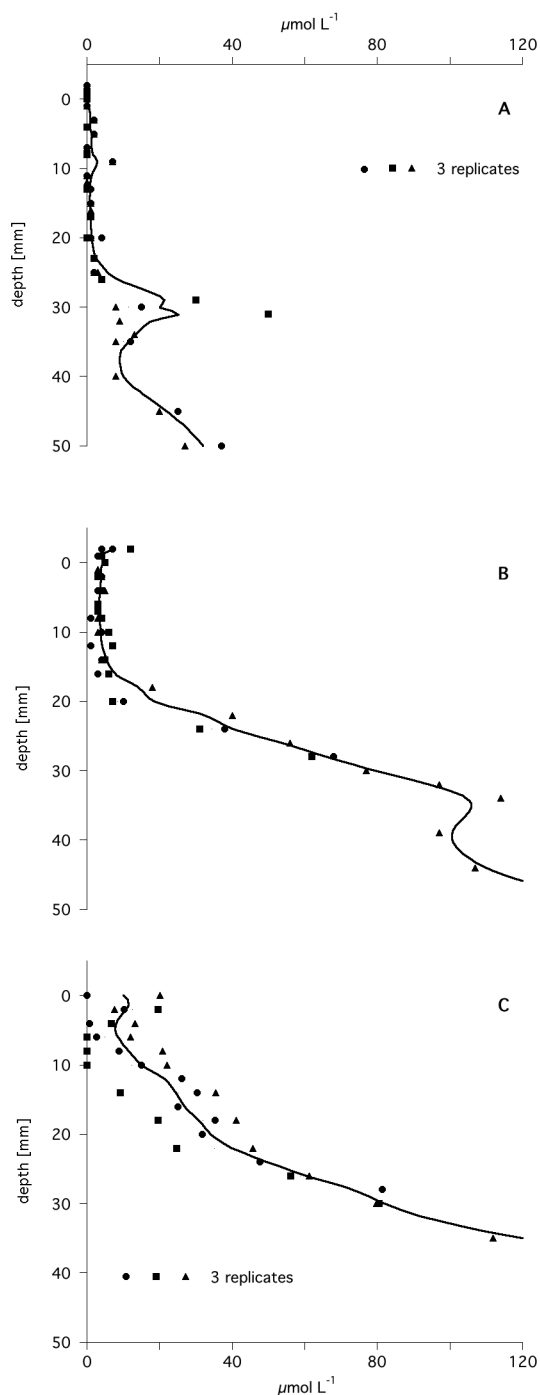


Fig. 7. Examples of methane profiles in littoral sediment in February 2005 (A) and in August 2004 (B) and in profundal sediment in August 2004 (C). Different symbols indicate replicate measurements, and the line indicates the mean of the replicates.

of $2 \mu\text{mol L}^{-1}$, so these values were excluded from the subsequent calculations. In the upper 30 mm, clear differences between the parallel measurements became evident (Figure 8A). High variability in the upper 30 mm is demonstrated by plotting the variation coefficients (standard deviation/mean * 100)

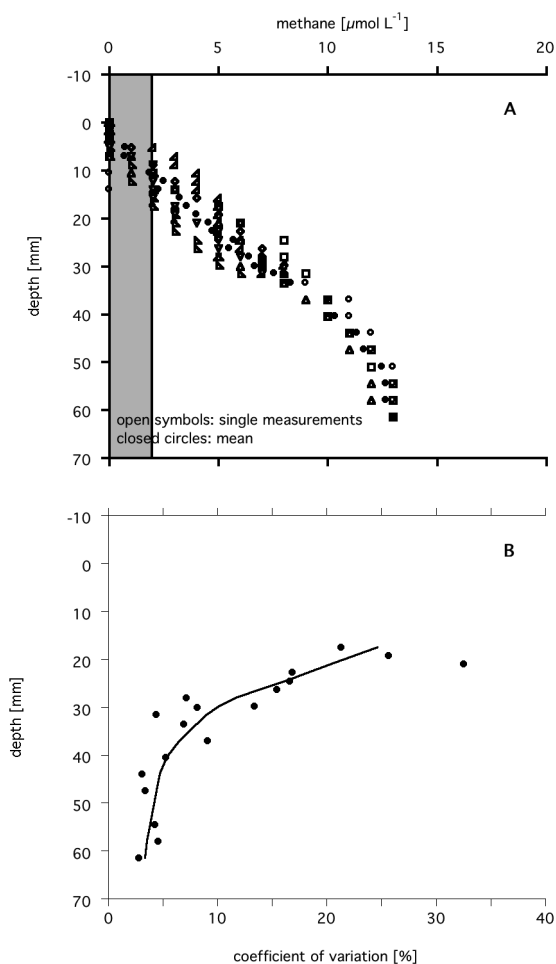


Fig. 8. Repeated measurements in one littoral sediment core (A), $n = 7$ for the upper 30 mm and $n = 3$ for the lower 30 mm, all 5 mm apart. The shaded area indicates the detection limit of $2 \mu\text{mol L}^{-1}$. The coefficient of variation (standard deviation/mean * 100) is shown for the same measurements (B).

versus depth (Figure 8B). In the lower 30 mm, methane was distributed more homogeneously within the core, as shown by lower variation coefficients. With a power test and the standard deviation of the data described above, we calculated how many parallel measurements are necessary to detect a difference of $2 \mu\text{mol L}^{-1}$ between 2 sediment cores. It turned out that each core has to be analyzed 5 times over the upper 30 mm ($\beta = 0.85$, $\alpha = 0.05$, standard deviation = 0.91), but only 3 times over the lower 30 mm.

Sediments at our littoral study site are highly variable in methane content, but with conventional headspace analysis this variability is difficult to analyze (Bussmann 2005). Parallel measurements with the methane sensor within one sediment core yielded only slight differences between the single profiles, but realistic values can be obtained with 3 to 5 parallel measurements per core. However, with the higher spatial resolution it was also possible to localize distinct areas with higher methane concentrations (Figure 7B, square profile).

Discussion

Based on the gas diffusion sensor originally introduced by Rothfuss and Conrad (1994), a methane sensor system was developed in which the sensor is directly connected to the flame ionization detector of a gas chromatograph. Calibration and tests showed that the newly designed sensor is a reliable tool for fine-scale and high-resolution methane measurements. With this sensor, we were able to measure methane profiles in freshwater sediment down to a limit of 5 to 6 cm at high spatial (1.5 mm) and high temporal (3 minutes per measurement) resolution. The detection limit in sediment was $2 \mu\text{M}$ methane. The system was reliable and reproducible, and one sensor can be used for several months.

The spatial resolution of diffusion-based microsensors depends on the size of the membrane area. The membrane area and the spatial resolution of the systems of Rothfuss and Conrad (1994) and Lloyd et al. (1996) lie within the same range as ours. Only the microscale biosensor of Damgaard and Revsbech (1997) has a higher spatial resolution (tip diameter of $50 \mu\text{m}$). The temporal resolution of our system is given with 3 min of diffusion, but at higher concentrations the diffusion time could be reduced and faster measurement will be possible. Analysis with a photoacoustic detector also requires only 3 min (Rothfuss et al. 1996), whereas analysis with a mass spectrometer requires 20 min in the sediment (Lloyd et al. 1996). Again the microscale biosensor has the highest temporal resolution (99% response time of 20 s) (Damgaard and Revsbech 1997). In contrast to the biosensor, in our system there was no interference from other substances, such as carbon dioxide or sulfide. Although the spatial and temporal resolutions of the microscale biosensor are superior, this sensor is very sensitive and its application has been very limited. Our detection limit ($2 \mu\text{M}$) was much lower than that of the original sensor with $20 \mu\text{M}$ (Rothfuss and Conrad 1994) and is close to the $1 \mu\text{M}$ sensitivity of the biosensor (Damgaard and Revsbech 1997). The advantage of our system is the direct connection of the sensor to a flame ionization detector of a gas chromatograph. Such a flame ionization detector is readily available, does not require large laboratory equipment, and can even be deployed in the field.

Our methane sensor allowed us to do replicate measurements of methane within 1 core. We observed that methane distribution was heterogeneous in the top 30 mm but became more homogeneous from 30 to 60 mm depth. In most studies, methane profiles are based on single measurements, so not much is known about the spatial variability of methane distribution in sediments. Replicate measurements by Roy et al. (1996) and Damgaard and Revsbech (1997) showed only small variations, but this may be due to the high methane concentrations observed in their cores. Only with the precursor of our sensor it was shown that the variability of methane distribution in anoxic paddy soil increased with depth due to the preponderance of methane bubbles in deeper soil layers (Rothfuss

and Conrad 1998). The high spatial variability of methane in the littoral zone of a lake is probably influenced by the quality and distribution of detritus in the deeper sediment (Kankaala et al. 2003).

Our methane profiles in freshwater sediment at a spatial resolution of 1.5 mm showed a clear zonation of the methane distribution. In the littoral cores, the upper 10 to 20 mm were devoid of methane. In summer, this zone was followed by a steep increase of methane with depth, whereas this increase was not so pronounced in winter. Microscale profiles of nitrate, nitrite, and oxygen have been used in several studies to calculate their diffusive fluxes and the respective production or consumption rates (Meyer et al. 2005; Nicolaisen et al. 2004). In these studies, the calculated activities have been related to measured potential activities as well as to the abundance of the bacteria involved. Until now, determination of the methane oxidation rate had to rely on the tedious $^{14}\text{CH}_4$ injection technique or on measurements in sediment slurries (Treude et al. 2005). With both these techniques, the spatial resolution is within the centimeter range. With the procedure of Berg et al. (1998), it should be possible to exactly locate the zones of methane production and especially of methane oxidation. The high-resolution methane profiles developed here allow calculations of diffusive fluxes from the sediment into the water: $1 \mu\text{mol m}^{-2} \text{d}^{-1}$ for the littoral and $70 \mu\text{mol m}^{-2} \text{d}^{-1}$ for the profundal cores. Calculations and measurements of the diffusive methane flux for sediments from Lake Constance have been done before. For the profundal cores, our calculated flux is within the range of previously measured fluxes (Frenzel et al. 1990), whereas for the littoral cores, much higher values have been measured and calculated from conventionally determined methane profiles (Bosse et al. 1993; Bussmann 2005).

It has to be kept in mind, however, that the methane profiles presented in this study are still not *in situ* profiles. The sediment cores were kept at near-*in situ* conditions as done in other studies, but as our study site is exposed to waves and water currents, fluid exchange between the sediment and the overlying water cannot be taken into account (Precht and Huettel 2003). Further developments should aim to connect the sensor described in this study to a methane detector that can be operated under water to obtain real *in situ* methane profiles.

Future application of this methane sensor will certainly lead to much more detailed insight into the dynamics and qualitative changes of methane concentrations in sediments and will deepen our understanding of the methane cycle in these ecosystems.

Comments and recommendations

For routine measurements, the total system has now been automated using microelectric valves (4port-2ways and 3port-2ways valves with microelectric valve actuator from Vici Valco, Schenk, Switzerland) and a micromanipulator controlled by a computer. With these modifications, the system can run autonomously and sample multiple profiles without

further handling. With this improvement, the sensor can be used to record numerous methane profiles from a single sediment core within a few hours, and thus obtain a 3-dimensional picture of methane distribution, which could provide a more realistic basis for calculation of methane fluxes from sediments than the few data sets on poorly resolved methane gradients could do so far.

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