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A laboratory microcosm for simultaneous gas and nutrient flux measurements in sediments

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We developed a laboratory microcosm to study simultaneously the dynamics of gases and nutrients in sediments. With the microcosm, intact sediment cores were incubated under continuous water flow. The incubation system was constructed so that diffusion and ebullition of gases could be measured separately. Here we show results from an experiment conducted with littoral, shallow and deep profundal sediments of Lake Kevätön using oxic and anoxic water flow. The fluxes of important greenhouse gases (methane, carbon dioxide, and nitrous oxide) and nutrients (phosphorus, ammonium, nitrite and nitrate) were determined. Deep profundal sediments released carbon gases, methane and carbon dioxide, and nutrients, ammonium and phosphorus, especially in anoxic conditions. Also the littoral sediment was important source of methane. Nitrous oxide, nitrite and nitrate were produced in the shallow profundal sediments with the oxic flow. Our microcosm was a promising tool to differentiate gas and nutrient dynamics between sediments under controlled conditions.

Introduction

Sediment processes are important in biogeochemical cycles of elements in aquatic environments. Sediments are reservoirs of autochthonous and allochthonous material and are, therefore, important sites of organic matter mineralization. Degradation in sediments produces gases, methane (CH_4) and carbon dioxide (CO_2), and regenerates nutrients, nitrogen (N) and phosphorus (P). Microbial driven mineralization of phosphorus can be the main reason for internal nutrient loading maintaining long-term eutrophication of lakes (Matinvesi 1992). In the course of eutrophication, the amount of degradeable organic matter and the production of gases from

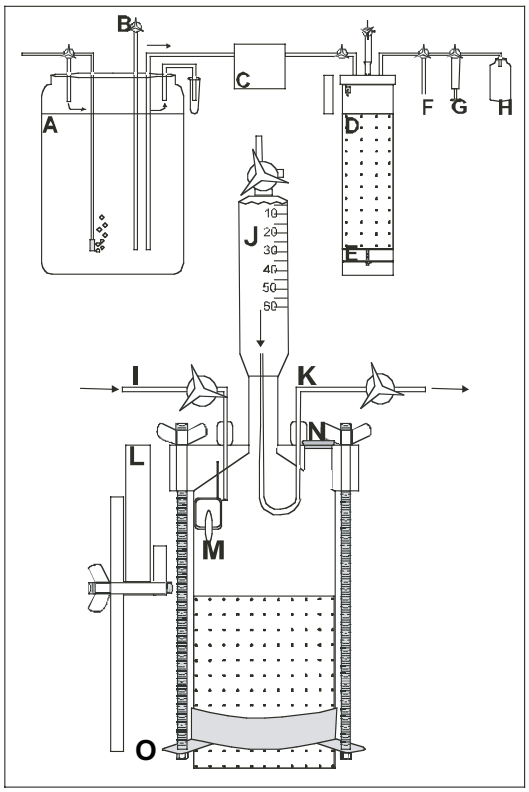

Fig. 1. The continuous water flow system (A to H) with a gas and water tight lid (I to O). Components: reservoir (A), sampling outlet for reservoir water (B), peristaltic pump (C), sediment core (D), bottom cap (E), outflow to drain (F), water sampling with a syringe (G), water sampling with a flask (H), inflow (I), gas trap (J), outflow (K), magnetic stirrer (L), rotating magnet (M), port for electrodes (N), removable clamp (O). With kind permission from Kluver Academic Publishers (Liikanen *et al.* 2002b)

mineralization increase (Casper 1992). Some of these gases, e.g. carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), are important greenhouse gases in the atmosphere. Methane also has a particularly significant role in freshwater ecosystems. Its oxidation contributes to anoxia, and the ebullition of methane leads to turbation in the sediments, enhancing the transport of nutrients to the upper water column, where nutrients are available for primary producers (Boström *et al.* 1988). Thus sediment gas and nutrient dynamics have importance not only for regional water quality but also are contributors to the atmospheric gas content.

Sediment–water interactions and microbial/chemical processes in sediments are difficult to quantify *in situ*. Intact sediment cores taken for controlled laboratory experiments offer a possibility to study the processes and material flows in sediments. Laboratory microcosms for intact sediments have been successfully used to study e.g. nutrient dynamics (e.g. Søndergaard 1989, Andersen and Jensen 1992, Binnerub *et al.* 1992), microbiology (e.g. Wagner-Döbler *et al.* 1992) and ecotoxicology (e.g. Hedtke 1984, Johnson 1986) of sediments. Also sediment gas fluxes, most commonly N_2 production (Seitzinger 1993, van Luijn *et al.* 1999) and O_2 consumption (Bowman and Delfino 1980, Nishio *et al.* 1982, Andersen and Jensen 1992), have been determined with the microcosms experiments. However, these experimental setups for gas studies have not applied dynamic constant flow-through technique and/or have not quantified both diffusion and ebullition of gases. We present here a laboratory microcosm with a continuous water flow technique for use with intact sediment cores to study diffusion and ebullition of gases, nutrient fluxes, and the interactions between gas and nutrient dynamics in sediments. We show the results of an experiment to determine the effects of oxygen conditions on gas and nutrient dynamics in sediments of a eutrophic lake.

Materials and methods

A laboratory microcosm

The microcosm experimental system is shown in Fig. 1. Intact sediment cores and a control core without sediment were housed in a dark temperature controlled room and were incubated with a continuous water flow. The constructed system permits the separation of the ebullitive gas fluxes from the fluxes of dissolved gases (diffusional flux). The sediments can be incubated at different temperatures, with oxic or anoxic water flows, and with water having different nutrient loads.

Sediments were collected directly into the incubation cores (D) (transparent acrylic tube, ID 94 mm, OD 100 mm, height 650 mm) fitted into the samplers. Soft profundal sediments were taken with a Limnos sediment sam-

pler (Limnos Oy, Turku, Finland) and littoral sediments with a stainless steel piston core sampler with a sharpened cutting edge.

The position of sediment in the core was adjusted by a moveable water-tight bottom cap (E). The core was sealed with a water and gas tight polyethene lid (I-O). The inlet (I) and outlet (K) of water were equipped with stainless steel connectors through the lid. The lid contained also a port (N) (diameter, 22 mm), which was sealed with a butyl rubber stopper. A gas trap (J) was constructed from a 60 ml polypropene syringe (Terumo Europe, Leuven, Belgium) equipped with a three way stopcock (Codan Steritex, Hoejvangen, Denmark) sited into the center of the hollowed lid. The gas trap had been first filled with water and during incubation water was replaced by gas evolved from the sediment.

Water was pumped from a reservoir (A) (volume, 80 l) continuously into the cores with a peristaltic pump (C) (IPC-24, Ismatec®, Switzerland). In the reservoir, the water was either in equilibrium with air (oxic water, ~10 mg O_2 l^{-1}) or was deoxygenated with N_2 (anoxic water, < 0.7 mg O_2 l^{-1}). The overlying water in the core was mixed slowly with a rotating Teflon-coated magnet (M). The magnet received momentum from a magnetic stirrer (L) (IKA Color Squid, IKA Labortechnik, Germany) placed close to the outer wall of the tube. The materials used in the tubing were impermeable to gases, i.e. Tygon (Tygon® Standard R3603, ID 0.95 mm, Ismatec®, Swizerland) in the pump, otherwise PVC (ID 3 mm, OD 5 mm), and nylon in the joints (Kjeldsen 1993).

Water samples (20–30 ml) for dissolved gas analysis were taken with 60 ml syringes (G) equipped with three-way stopcocks, and samples for nutrient analysis were collected in flasks (H) from the outflow. The rate of water flow from the cores was measured in each sampling. The fluxes of nutrients and dissolved gases (μg or mg m^{-2} d^{-1}) from sediments were calculated from the concentration differences in outflowing water between the control (no sediment) and sediment cores using flow rates and sediment surface area.

Accumulated bubble gases were collected from the gas traps (J) with syringes equipped with tree way stopcocks. The volumes and concentrations of the bubble gases were determined. The ebullited gas flux rates (μg or mg m^{-2} d^{-1}) were calculated by dividing the amount of gases collected with the incubation time and sediment surface area.

The sediment oxygen consumption (SOC) was determined during the oxic water flow. The O_2 concentration in the overlying water was measured with an oxygen electrode inserted through the port (N) in the lid. The SOC (mg O_2 m^{-2} d^{-1}) was calculated from the difference in the O_2 concentration of water between the control and sediment cores using flow rates and sediment surface area.

An experiment with sediments from a eutrophic lake

The microcosm was applied to measure fluxes of greenhouse gases and nutrients from sediments of a eutrophic lake. Triplicate sediment cores (sediment height 30–40 cm) were taken from littoral (1-m depth middle infralittoral growing *Phragmites australis* and *Nuphar lutea*), and shallow (4-m depth) and deep profundal (9-m depth) of Lake Kevätön (63°6´N, 27°37´E) in June 1998. At the time of sampling, the hypolimnion was oxic and the sediments were, therefore, incubated first with the oxic water flow for three weeks, and then with the anoxic flow for two weeks. The incubation temperature was 15 °C similarly to the temperature in the hypolimnion of Lake Kevätön (Table 1).

The water used in this study was bank filtered and chemically [$Al_2(SO_4)_3$] coagulated freshwater from the Kuopio waterworks (Kuopio, Finland) (0.1 μM tot-P, 1 μM NH_4^+-N, 3 μM (NO_2^- + NO_3^-)-N, 230 μM SO_4^{2-}, < 20μM Fe, and pH 6.7). Water from Lake Kevätön (hypolimnetic water at 9-m depth in 1998: 1–2.3 μM tot-P, 1–130 μM NH_4^+-N, 0–7 μM (NO_2^- + NO_3^-)-N, 30–67 μM SO_4^{2-}, and 6–146 μM Fe, and pH 6.8–7.5) was not used since the quality of lake water would have varied during the incubation of one month and lake water should have been filtrated before usage. The volume of the overlying water in the core was about 550 ml and the pumping rate was 50 ml h^{-1}

resulting the water turnover time of 11 h.

The fluxes of dissolved gases, methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O), were measured 3–5 times a week. The gas evolved was collected when 3–10 ml was accumulated in the gas traps (J). Gas samples were analysed within 24 h from the sampling. The gas concentrations in the water were analysed with a headspace equilibration technique (McAuliffe 1971). Water samples of 20–30 ml were preserved with sulphuric acid (1 ml H_2SO_4, 20% v/v) immediately after the sampling. Water was equilibrated with added nitrogen (30–40 ml) by shaking vigorously for 3 minutes. The dissolved gas concentrations in the water samples were calculated from the headspace gas concentrations according to Henry's law using the values from Lide and Fredikse (1995). The CH_4, CO_2, and N_2O concentrations were determined with two gas chromatographs (Hewlett Packard [Palo Alto, CA] 5890 Series II) equipped with a flame ionization detector for CH_4, a thermal conductivity detector for CO_2 and CH_4 (> 1000 ppm CH_4), and an electron capture detector for N_2O (*see* details Nykänen *et al.* 1995).

The fluxes of nutrients, total phosphorus (tot-P), ammonium (NH_4^+), nitrite and nitrate ($NO_2^- + NO_3^-$), and non-volatile organic carbon (NVOC) were determined at the end of incubation periods. Analyses were done from the samples stored at –20 °C. Combined nitrite and nitrate were determined with FIA (flow injection analysis) method (Lachat [Milwaukee, WI] Quick Chem 8000) according to the standard SFS-EN ISO 13395 (SFS Standardization 1997) and the instructions of the device manufacturer. Ammonium was determined photometrically according to the standard SFS 3032 (SFS Standardization 1976). Total phosphorus was meas-

Table 1. Characteristics of the microcosm sediments and hypolimnetic water in Lake Kevätön at the time of sampling. Water temperatures and O_2 concentrations in hypolimnion (0.5–1 m above the sediment surface). Contents of carbon and nitrogen in the sediments and C/N ratio in the sediment layers of 0–2 cm.

Site	Lake water		Sediment		
	T °C	O_2 mg O_2 l^{-1}	Carbon mg C g^{-1}	Nitrogen mg N g^{-1}	C/N
Littoral	20	n.d.	n.d.	n.d.	n.d.
Shallow profun.	15	10	55	11	5
Deep profun.	13	9.6	87	16	5.4

n.d. = not determined.

Table 2. Conditions in the microcosms. Overlying water O_2 concentration and pH, sediment oxygen consumption (SOC) and redox potential, the averages and standard error of the means in parentheses.

Sediment	Water flow	O_2 conc[a] (mg O_2 l^{-1})	SOC (mg O_2 m^{-2} d^{-1})	redox[b] (mV)	pH[a]
Littoral	Oxic	4.6 (0.9)	530 (150)	130 (11)	6.2 (0.1)
	Anoxic	0.4 (0.2)	n.d.	–62 (17)	6.7 (0.1)
Shallow profun.	Oxic	4.9 (0.1)	490 (27)	37 (5)	6.3 (0.1)
	Anoxic	0.1 (0.1)	n.d.	–100 (2)	7.1 (0.1)
Deep profun.	Oxic	2.5 (0.3)	890 (39)	–43 (6)	6.5 (0.1)
	Anoxic	0.0 (0.0)	n.d.	–140 (7)	7.1 (0.1)
Control	Oxic	8.1	n.d.	n.d.	n.d.
	Anoxic	1.2	n.d.	n.d.	n.d.

[a] Measured ~ 1 cm above the sediment surface.
[b] Measured ~ 0.5 cm below the sediment surface.
n.d. = not determined.

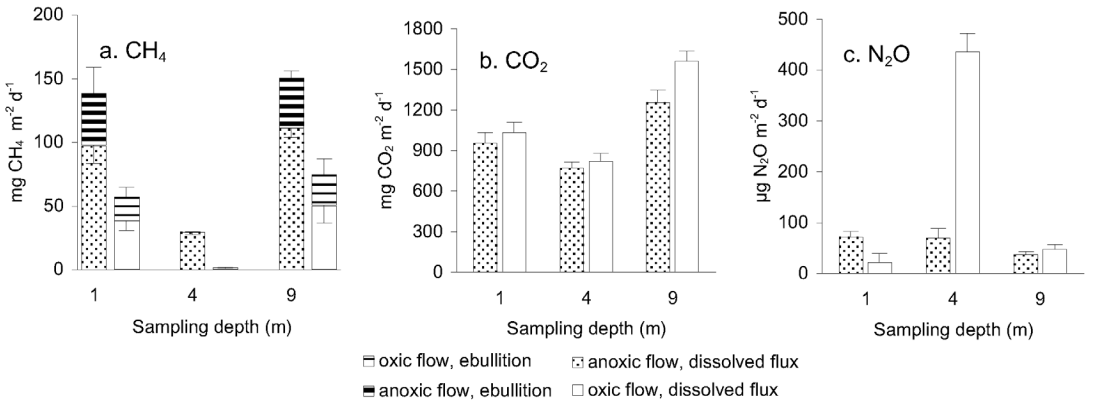

Fig. 2. The dissolved and ebullitive (**a**) CH_4, (**b**) CO_2, and (**c**) N_2O fluxes from the sediments determined with the oxic or anoxic flows. Averages and standard error of means.

ured photometrically according to the standard SFS 3026 (SFS Standardization 1986). Non-volatile organic carbon (dissolved C, excluding volatile C-compounds) was analyzed from water samples filtrated through 0.22 μM membrane (Millipore, USA) with Shimadzu TOC-5000 Analyzer (Shimadzu Corp., Japan).

Overlying water O_2 concentration and pH, sediment O_2 consumption (SOC), and redox potential were determined once at the end of the incubations (Microprocessor pH meter pH320, WTW, Germany, with Hamilton pH electrode and with InLab®501 redox electrode, Mettler Toledo, Switzerland; Dissolved Oxygen Meter Oxi 330 with Dissolved Oxygen Probe CellOx 325, WTW, Germany). After the experiment, carbon (C) and nitrogen (N) contents of the microcosm sediments in the layer of 0–2 cm were determined with a LECO CHN-600 element analyzer from the sediment samples stored at –20 °C.

Results

Lake and sediment characteristics

At the time of sampling, hypolimnia of Lake Kevätön was oxic and temperature varied from 13 to 20 °C (Table 1). Sediment carbon and nitrogen contents were higher in the deep profundal than in the shallow profundal sediments (Table 1).

Conditions in the microcosms

Anaerobic conditions were achieved with the anoxic flow, when the overlying water O_2 concentration was below 0.3 mg O_2 l^{-1} (Table 2). With the oxic flow, sediment oxygen consumption (SOC) regulated O_2 concentration in the overlying water from 2.3 to 5.6 mg O_2 l^{-1} (Table 2). The SOC was highest in the deep profundal sediments rich in organic carbon. Sediment redox potential (0.5 cm below the surface) was lower and pH of the overlying water was slightly higher with the anoxic flow.

Gas fluxes

The CH_4 fluxes were greatest from the deep profundal and the littoral sediments (Fig. 2a). In these sediments, ebullition accounted for 32% ± 5% (mean ± S.E.) of the total CH_4 fluxes. With the oxic flow, the total CH_4 fluxes were reduced by 68% ± 14% (mean ± S.E.), the diffusive CH_4 flux was reduced by 70% ± 12% and the ebullited CH_4 by 46% ± 8%. The dissolved CH_4 flux was linear over time under oxic conditions and increased sharply at the onset of anoxia (Fig. 3). The CO_2 fluxes were greatest from the deep profundal sediments (Fig. 2b). The differences between the CO_2 fluxes with the anoxic and oxic flows were minor. Highest CO_2 fluxes were measured in the beginning of the incubation (Fig. 3). Significant N_2O fluxes were

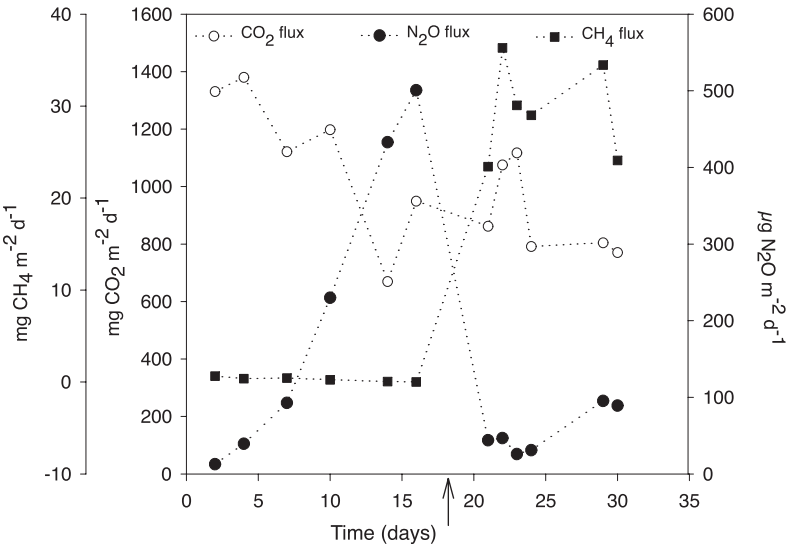

Fig. 3. Fluxes of dissolved gases (CH_4, CO_2, and N_2O) from a shallow profundal sediment during the experiment. The arrow indicates the change from the oxic to the anoxic water flow.

observed only from the shallow profundal sediments incubated with the oxic flow (Fig. 2c). The N_2O flux increased over time with the oxic flow (Fig. 3).

Nutrient fluxes

The deep profundal sediment with highest carbon gas production was the greatest sources of P and NH_4^+ (Fig. 4a and b). The presence of O_2 in the water reduced the release of P and NH_4^+ from the sediments. The NO_2^- and NO_3^- were produced in the shallow profundal sediments with the oxic flow, other sediments consumed NO_2^- and NO_3^- (Fig. 4c). The sediments were sources for organic carbon with the anoxic flow but consumed organic carbon with the oxic flow (Fig. 4d).

Discussion

Evaluation of the microcosm

The microcosm described here is a promising tool for studying simultaneously both gas and nutrient dynamics in sediments and allows the separation of ebullition and diffusion of gases. Previous microcosms studies have not quantified ebullition of gases and have not measured gas fluxes over long period of time with dynamic flow-through systems (Seitzinger 1993, van Luijn *et al*. 1999). Also in our microcosm, the ebullition might have been underestimated, since some bubbles, probably containing mainly CH_4, were trapped to sediments and this gas production was neglected. Most commonly, the diffusive material fluxes between sediment and water have been determined indirectly from depth gradients according to Fick's law. However, these diffusive flux calculations make rough assumptions on diffusivity and tortousity, miss the ebullition of gases and neglect the turbation in sediments. On the other hand, continuous water flow applied in the microcosm may have increased the diffusion of gases and nutrients from the sediments to continuously changing water, because there is no development of stagnation and build up of gradient, a normal situation in lakes, which decreases diffusion rate from sediments. Therefore, with the microcosm we cannot determine absolute gas and nutrients fluxes *in situ*. However, the microcosm studies show relative differences in gas and nutrient dynamics between various sediments.

The microcosm allows a good control of environmental parameters. Using *in situ* approaches, it is difficult to find the environmental factors controlling the nutrient and gas dynamics in

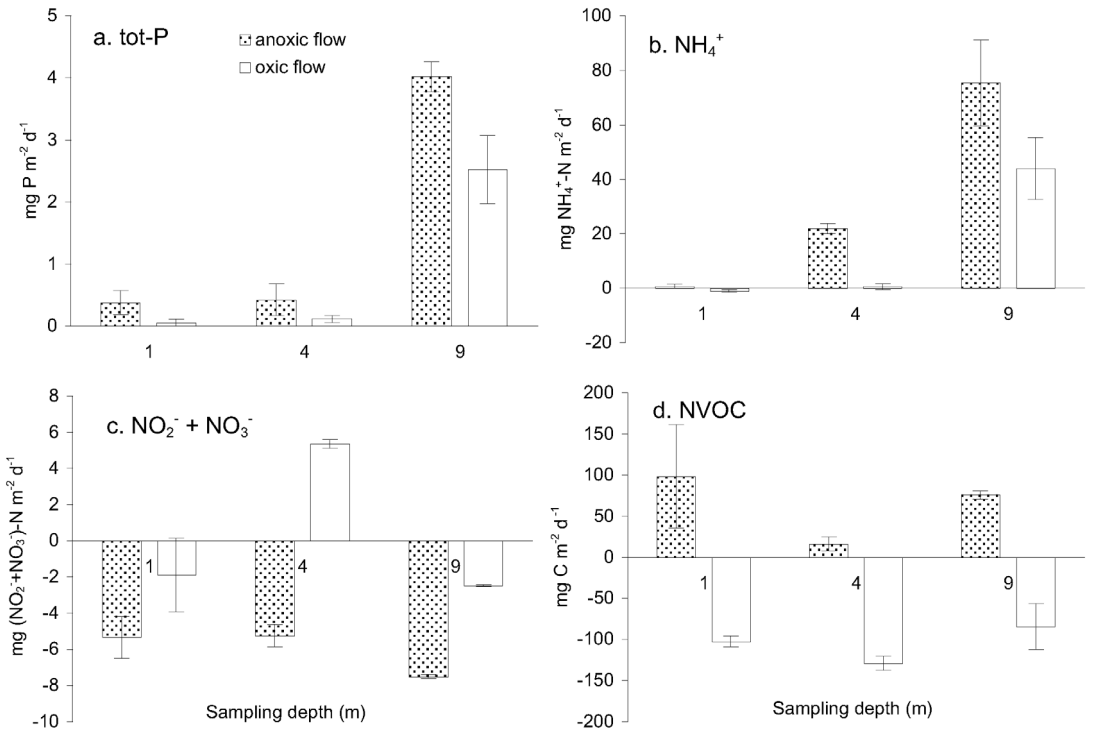

Fig. 4. Fluxes of (**a**) tot-P, (**b**) NH_4^+, (**c**) NO_2^- + NO_3^-, (**d**) NVOC from the sediments determined with the oxic or anoxic flows. Averages and standard error of means.

different sediments. Here we were able to adjust constant temperature and both anoxic and oxic conditions to the microcosms. With the anoxic flow, overlying water O_2 concentration was always below 0.3 and with the oxic flow above 2.3 mg O_2 l^{-1}.

The undisturbed, layered sediment core with a dynamic continuous water flow seemed to be stable throughout the experiment. The gas fluxes did not vary greatly from day to day. The CH_4 production was stable through the oxic and anoxic incubations. However, some decrease in the CO_2 production occurred during the run, probably because the sediments were running out of labile organic material. Instead, the N_2O production increased linearly with time during the oxic incubation, and a steady-state N_2O production was not obtained within three weeks oxic incubation. In the course of oxic run, nitrification activity and sediment NO_3^- content has probably increased, which has further stimulated denitirification and N_2O production deeper in anoxic sediments. In sediments, NO_3^- availability highly regulates the N_2O production (*see* e.g. Knowles 1979).

Mineralization and gas and nutrient dynamics in the sediments

Highest microbial activities and mineralization rates, i.e. production of CH_4 and CO_2 and consumption of O_2, were measured in the littoral and deep profundal sediments. In these sediments, the content of organic matter has probably been greater than in the shallow profundal sediments. The deep profundal sediments had received organic material from other parts of the lake and in the littoral, plants had generated fresh organic material to sediment. Simultaneous measurement of CH_4 and CO_2 production together with O_2 consumption gave information about the aerobic and anaerobic degradation in sediments. Oxygen is consumed in aerobic

respiration, whereas CO_2 is produced both in aerobic respiration and various anaerobic processes. If the molar ratio of produced CO_2 to consumed O_2 (respiratory quotient) is greater than unity, the CO_2 is also derived from anaerobic mieralization (Rich 1975). In these sediments, respiratory quotients were 1.5, 1.3, and 1.3 in the littoral, shallow profundal, and deep profundal sediments, respectively. Therefore, with the oxic flow, also anaerobic processes produced some CO_2. With the anoxic flow, the CO_2 production was similar to the CO_2 production with oxic flow, and in addition, significant amounts of CH_4 were produced. Thus organic matter was mineralized in the sediments at the same rate whether or not O_2 was available in overlying water indicating the importance of anaerobic mineralization in the sediments. In our experiment, sulfate reduction, important in anaerobic mineralization processes, was probably stimulated since test water SO_4^{2-} concentration of 230 μM was 3–8 times higher than the *in situ* concentrations, from 30 to 67 μM, in Lake Kevätön. However, the SO_4^{2-} concentration used in this experiment is close to concentrations from 0 to 200 μM generally found from freshwaters (Capone and Kiene 1988). Sulfate reduction is known to inhibit CH_4 production, since more energy yielding sulfate reducers can compete methanogens for organic substrates (Capone and Kiene 1988). Therefore, CH_4 release from the sediments might be underestimated. According to our previous results, concentration of 200 μM SO_4^{2-} slightly reduces CH_4 release from the shallow profundal sediments with low organic matter content but does not inhibit CH_4 production in the organic deep profundal sediments (the effects of SO_4^{2-} were not studied in the littoral sediments) (Liikanen *et al.* 2002a).

Methane production, its ebullition and oxidation were significant in the deep profundal and littoral sediments. In these sediments, ebullition was an important part of the total CH_4 flux both with anoxic and oxic flows. Especially in eutrophic lakes, significant amount of CH_4 is released by ebullition (Takita and Sakamoto 1993, Nakamura *et al.* 1999). It is well known that CH_4 in bubbles is protected against activity of methane-oxidizing microbes (Chanton and Martens 1988). The evolved CH_4 in bubbles is released directly from anoxic basins to the atmosphere, where CH_4 is an important greenhouse gas contributing the global warming. However, there was a significant reduction in the total CH_4 fluxes with the oxic flow. The difference in the CH_4 fluxes between oxic and anoxic incubations is used as a measure of the CH_4 oxidation (van Luijn *et al.* 1999). This approach assumes that methanogenesis deeper in the anaerobic sediment is not greatly inhibited with the oxic water flow. Thus in the littoral, shallow and deep profundal 81, 28, and 75 mg CH_4 m^{-2} d^{-1} would have been oxidized, respectively. These CH_4 oxidation rates would have consumed 260, 90, and 240 mg O_2 m^{-2} d^{-1} in the littoral, shallow and deep profundal sediments (assuming that molar ratio of O_2 consumed to CH_4 oxidized is 1.6, Joergensen and Degn 1983), corresponding to 49%, 18%, and 27% of the total O_2 consumption in these sediments, respectively. Similarly Sweerts *et al.* (1996) found that CH_4 oxidation consumed from 2% to 64% of the total O_2 consumption. The oxidation of CH_4 is one of the most important processes consuming O_2 in freshwater sediments (van Lujin *et al.* 1999).

In addition to the exchange of gaseous carbon compounds, were observed the exchange of dissolved organic carbon between sediment and water. The aerobic sediment of a eutrophic lake consumed organic carbon from water, whereas under anaerobic conditions, organic carbon was released from the sediment. Thus, during O_2 deficiency, the sediments released not only CH_4 and CO_2 but also organic matter to the water.

Mineralization and O_2 availability were the key factors regulating nutrient dynamics in the sediments. The deep profundal sediment with the highest mineralization and O_2 consumption rates also had the greatest release of P and NH_4^+, implying that microbial processes were closely involved in P release (Sinke *et al.* 1990). Microbial degradation mobilized nutrients from organic matter and consumed O_2 lowering sediment redox potentials. It is well known that in reducing conditions P is liberated from iron(hydr)oxyphosphate precipitate when Fe (III) reduces to Fe (II) (e.g. Boström *et al.* 1988). Ebullition of CH_4 caused turbation in soft deep profundal sediments and may have transformed nutrients from sediment pore water to overlying

water (Boström *et al*. 1988). The deep profundal sediment was an important reservoir of nutrients, whereas the littoral sediment, which also showed high mineralization, was not the source of nutrients. The shallow porfundal sediment with low O_2 consumption was able to produce NO_2^- and NO_3^- for denitrification and N_2O production. In deep profundal sediments with high O_2 demand, nitrification and associated N_2O production were probably limited by the lack of O_2. It is well known that in freshwater sediments, low availability of NO_3^- limits denitrification, which therefore is closely coupled to nitrification (e.g. Knowles 1979)

The experiment here revealed the close association of gas and nutrient dynamics in freshwater sediments. Sediment oxygen status had great impact on both gas and nutrient fluxes between sediment and water. With stable and controlled conditions the microcosm study denoted the differences between the sediments within the eutrophic lake.

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