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Biogeochemistry of methane in the permanently ice-covered Lake Untersee, central Dronning Maud Land, East Antarctica

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Abstract

We found unprecedentedly high abundances of microbially produced CH_4 in the anoxic deep waters of Lake Untersee, an oligotrophic, perennially ice-covered Antarctic freshwater lake. The maximum CH_4 concentration (approaching 21.8 ± 1.4 mmol L^{-1}) is one of the highest observed so far in a natural aquatic ecosystem. Although surficial lake sediments are the predominant source of CH_4 in Lake Untersee, methanogenesis occurs also within the anoxic waters. Radiocarbon labeling experiments show that H_2/CO_2 reduction is the predominant methanogenic pathway (90–100%) both in the sediments and the water column, whereas acetate is only a minor CH_4 precursor. This result is consistent with the stable carbon isotope fractionation between coexisting CH_4 and CO_2. In the water column, CH_4 is partly consumed by both aerobic and anaerobic microbial oxidation as evidenced by CH_4 concentration patterns, stable isotope analyses (^{13}C, ^{2}H), and ^{14}C-CH_4 assays. Dissimilatory sulfate reduction also occurs and peaks at 84 m water depth (1.83 μmol SO_4 L^{-1} d^{-1}). Intense methanogenesis in surficial lake sediments, diffusion of CH_4 from sediments to the water column, additional CH_4 production in the water column, gross CH_4 production higher than CH_4 consumption, and lack of mixing because of the permanent ice cover cause the exceptionally high CH_4 concentration in the lake. Our studies demonstrate that H_2/CO_2 reduction may sometimes be the major pathway of methanogenesis in low-sulfate freshwater environments even at low temperatures. This pathway is obviously more important in Antarctic lakes than hitherto assumed.

As an environmentally important greenhouse gas, methane (CH_4) plays a significant role in the global climatic system. Studies of CH_4 cycling in various environments is therefore of fundamental interest. Biological processes are the primary source (ca. 80–90%) of atmospheric CH_4 (e.g., Cicerone and Oremland 1988) and microbial CH_4 production (methanogenesis) occurs over a wide temperature range, below freezing up to boiling (Valentine and Boone 2000). Wetlands are thought to be the largest natural source of atmospheric CH_4 (Cicerone and Oremland 1988). Because about one half of all wetlands are located in high latitudes (tundra and taiga regions, boreal bogs, and fens), understanding of methane cycling in low-temperature terrestrial ecosystems is of great interest. Besides wetlands, there are a number of other natural methane sources, among them marine and lake sediments (Khalil and Rasmussen 1983; Cicerone and Oremland 1988). Methane is a key component of the carbon cycle in many aquatic environments, and it is an important end product in the anaerobic degradation of organic matter in marine and freshwater sediments, particularly in zones where more energy-yielding oxidants, such as sulfate, nitrate, or ferric iron, are depleted (Heyer 1990). Methanogenesis in freshwater systems may account for more than 50% of organic-matter degradation (Rudd and Hamilton 1978).

Methanogenic archaea are obligate anaerobes that convert only a limited number of simple substrates to CH_4 and CO_2.

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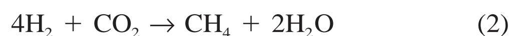
These substrates are produced during the decomposition of organic matter by a complex community of hydrolytic, fermentative, and acetogenic microorganisms (Conrad 1989). The most important methanogenic pathways involve conversion of simple organic substrates (e.g., acetate fermentation or acetoclastic methanogenesis) or carbon dioxide (i.e., the reduction of CO_2 by molecular hydrogen or hydrogenotrophic pathway):

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (1)$$

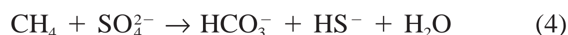


$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (2)$$

Other methanogenic pathways using substrates, such as formate, methanol, dimethyl sulfide, or methylamines, are less important quantitatively but can be important locally (Cicerone and Oremland 1988). The relative importance of the two primary methanogenic pathways depends on various environmental factors (presence of competitive consumers, in particular, sulfate-reducing bacteria, substrate availability, and temperature) (e.g., Winfrey and Zeikus 1979; Heyer 1990 and references therein; Schulz et al. 1997).

Oxidation of methane by aerobic methanotrophic bacteria (Eq. 3) (e.g., Rudd and Hamilton 1975) or anaerobic methanotrophic consortia (Eq. 4) (e.g., Reeburgh and Heggie 1977; Hoehler at al. 1994) effectively removes methane in water column and sediments and regulates CH_4 emission into the atmosphere. Hence, microbial consumption is the major methane sink:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \quad (3)$$



$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O \quad (4)$$

Production and consumption processes are reflected by the shape of concentration profiles of CH_4 (e.g., Barnes and Goldberg 1976; Winfrey and Zeikus 1979) and stable isotope compositions of CH_4 and CO_2 in marine and freshwater environments. Microbial processes of both CH_4 production and consumption result in stable isotope fractionations between substrates and products. Light isotopes (^1H, ^{12}C) are, in general, preferentially utilized in the related biochemical reactions (kinetic isotope effect) and will be consequently enriched in the end product. This discrimination is responsible for the observed depletion in ^{13}C of microbially produced CH_4 relative to the precursors (e.g., acetate and CO_2). The isotopic signature of the produced CH_4 is, moreover, controlled by the isotope composition of the primary carbon substrate.

The stable carbon isotope fractionation during methanogenesis is not constant (this holds also for stable hydrogen isotope fractionation), but varies as a function of the methanogenic pathway, substrate concentration, and process rate (e.g., Whiticar et al. 1986; Whiticar 1999; Conrad 2005). The magnitude of isotope discrimination can be expressed by the isotope separation factor ε, which is defined (e.g., for carbon) as follows:

$$\varepsilon_C\ (‰) \approx \delta^{13}C_{substrate} - \delta^{13}C_{product} \quad (5)$$

(for derivation of ε, *see,* e.g., Whiticar 1999; for definition of δ value, *see* Materials and Methods).

The compilation of stable carbon isotope data given by Conrad (2005) for both culture experiments and environmental studies shows a range of values, but the carbon isotope fractionation factors found for H_2/CO_2 reduction are significantly larger ($\varepsilon_C \approx$ 30–80‰) than those found for acetoclastic methanogenesis (ε_C up to 30‰). Factors such as microbial kinetics, temperature, maturity of organic matter, degree of eutrophication, and substrate availability may also affect the magnitude of isotope fractionation. Stable isotope fractionation associated with aerobic methane oxidation leads to ^{12}C depletion in the end product (CO_2) and ^{13}C- as well as ^2H-enrichment in the residual methane (Coleman et al. 1981). However, the isotope effects are normally less than those observed for methanogenesis ($\varepsilon_C \approx$ 5–30‰ according to culture experiments and model calculations) (*see* Whiticar 1999).

There have been efforts to estimate the methanogenic pathways from isotopic signatures. For example, plots of measured δ values of CH_4, CO_2, and ambient water were used (e.g., Whiticar et al. 1986; Hornibrook et al. 1997; Whiticar 1999) to characterize environments according to an apparent isotope fractionation factor α that is defined by

$$\alpha_C = (1{,}000 + \delta^{13}C_{CO_2})/(1{,}000 + \delta^{13}C_{CH_4}) \quad (6)$$

$$\alpha_H = (1{,}000 + \delta^2H_{H_2O})/(1{,}000 + \delta^2H_{CH_4}) \quad (7)$$

respectively. Thus, it is generally assumed that $\alpha_C < 1.055$ and $\alpha_C > 1.065$ are characteristic for environments dominated by acetoclastic and hydrogenotrophic methanogenesis, respectively (Whiticar 1999). As Conrad (2005) pointed out, such plots are helpful for a rapid but crude diagnosis of the predominant methanogenic pathway. Verification of the predominant process is achieved by conducting methanogenesis experiments using ^{14}C-labeled substrates such as [2-^{14}C]-acetate and ^{14}C-bicarbonate (e.g., Cappenberg and Prins 1974). Using both stable and radioisotope approaches provides a more thorough understanding of methane cycling in water ecosystems.

The biogeochemistry of methane cycling in lacustrine systems has mainly been studied in temperate zones. Little is known about the methane cycling in the extreme environment of Antarctic lakes. Such lakes serve as unique model systems for estimating the role of CH_4 in periglacial lacustrine environments during former glacial periods and for evaluating the importance of microbial biogeochemical processes in low-temperature aquatic ecosystems. In contrast with the polar regions of the Northern Hemisphere, there are only a few detailed studies on the occurrence and biogeochemistry of CH_4 in Antarctic lakes and ponds (e.g., Franzmann et al. 1991; Smith et al. 1993; Galchenko 1994).

Lake Untersee is one of the largest freshwater, perennially ice-covered lakes in East Antarctica. During an expedition in 1991–1992 (Wand et al. 1997), we found evidence of methanogenesis in a meromictic basin located in the southeastern part of the lake; the data led to a more detailed biogeochemical study of this exceptional lake site in February 1995. The extremely high CH_4 concentration (21.8 mmol L^{-1}) in the anoxic bottom water of such an oligotrophic freshwater lake was a surprising result. This water body thus represents a small natural gas deposit (dissolved CH_4 up to ~500 mL per liter of water). The methane anomaly was

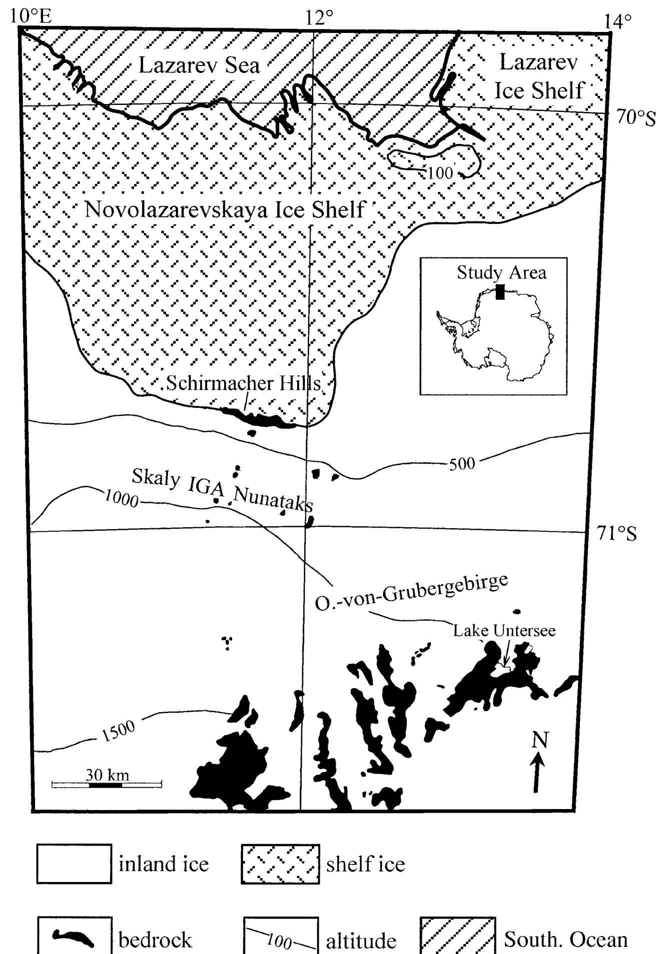


Fig. 1. Sketch map of the study area with the location of Lake Untersee, central Dronning Maud Land, East Antarctica. Altitude given in meters above sea level.

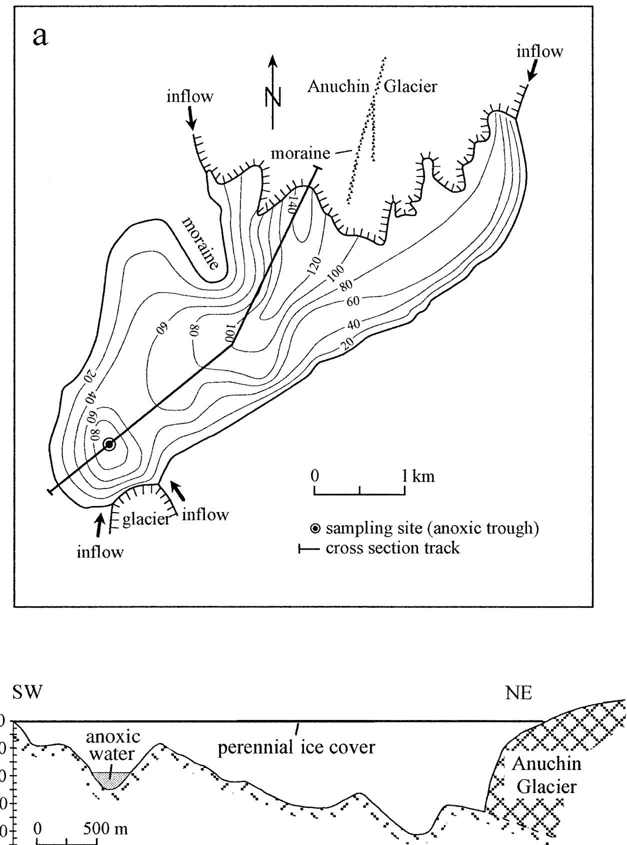


Fig. 2. (a) Bathymetric map and (b) cross section of the Lake Untersee basin.

accompanied by microbially driven processes such as CH_4 production, aerobic and anaerobic CH_4 oxidation, and sulfate reduction in the water column.

We present here new data on the concentrations and turnover of CH_4 in this Antarctic freshwater lake, demonstrating the activity of microbes under the permanently cold conditions prevailing on the Antarctic continent. This work furthers our understanding of the methane biogeochemistry of freshwater lakes in the interior of Antarctica, which are poorly known in contrast with the well-studied saline lakes in the Antarctic McMurdo Dry Valleys, the Bunger Hills, and Vestfold Hills.

Materials and methods

Physicochemical parameters and sampling—Sampling and physicochemical measurements for this study were carried out at the anoxic site in the southern part of the lake (Fig. 2) in February 1995. For these purposes, 20-cm-diameter holes were drilled through 2-m-thick ice using a Jiffy Power Drill. The physicochemical parameters temperature, pH, Eh, electrical conductivity, and dissolved oxygen content were measured in situ using probes each attached to a 100-m-long cable (WTW). Based on the results of the hydrographic profile, discrete depth intervals for subsequent water sampling were selected. Lake water was sampled at various depths using a 5-liter UWITEC water sampler without disturbing and mixing of the stratified water column. Subsamples for different analyses were immediately transferred via syringe to suitable vessels and, if necessary, filtered, fixed, or poisoned (e.g., for later stable isotope analyses) in the field. Samples for methane analysis were collected by syringe (10 mL) and injected into 30-mL evacuated and rubber-stoppered glass tubes containing $CdCl_2$ solution to precipitate sulfide. Surface sediments and near-bottom waters were collected into 7 cm (outer diameter) PVC tubes using an UWITEC gravity corer. Subsequently, the tubes were sectioned and samples for methane concentration measurements and radiotracer rate experiments were taken.

Geochemical measurements—Methane concentrations were measured using a head-space equilibration technique. From each depth interval, a 5-mL aliquot of lake water was removed from the water sampler using a syringe, taking caution to avoid gas bubbles, and was transferred to an evacuated 13-mL Hungate tube containing a pellet of NaOH. Gas pressure in tubes was equilibrated with atmosphere by inserting a needle. The tubes were vigorously shaken for 1 min before subsampling for analysis on a gas chromatograph

(GC). Gas samples were analyzed on site using a portable GC (XPM-4; Chromatograph Company) equipped with a flame ionization detector (FID), a 2-m stainless-steel column filled with Porapack Q, a thermoregulator, and a digital peak integrator. The instrument was calibrated in the field using 10, 10^2, 10^3, 10^4 ppm CH_4 standards prepared by mixing ultrapure CH_4 with 99.9996% N_2 in calibrated 1-liter glass bottles with butyl rubber septa and screw caps. The detection limit was 1 nmol L^{-1}. The accuracy, which is mostly influenced by sampling (e.g., degassing due to the transfer of the water samples from the depth to the surface, uptake of microbubbles with the syringe), was about 15%. For analysis, 0.5 mL of head-space gas was removed from sample tubes with a 1-mL gas-tight Hamilton syringe. For measuring methane concentrations, 10 mL of surface sediments were taken with a cut-end plastic syringe and placed into 1,000-mL glass bottles containing 100 mL of saturated NaCl with pH >10. Bottles were closed with butyl rubber septa and screw caps, shaken, and stored until analysis. CH_4 concentrations were measured using the same technique as for water.

Dissolved organic and inorganic carbon (DOC and DIC, respectively) in lake water were analyzed using a Shimadzu TOC-5000 total C analyzer. Sulfate concentrations were determined by ion chromatography. For acetate pool determinations, 20-mL lake-water samples were taken in 30-mL glass serum bottles (Bellco) containing a pellet of NaOH to halt biological activity. Samples were sealed with silicon septum and an aluminum crimp. Bottles were shaken to dissolve the NaOH pellet and were kept refrigerated until laboratory analysis. A vacuum distillation procedure was used to concentrate acetate from the water samples for GC analysis (Sørensen et al. 1981). The distillation efficiency was checked with ^{14}C-labeled acetate and was greater than 90%. The distillate was analyzed on a Varian 3700 GC equipped with FID at 200°C. Acetate was separated at 160°C on a 2-m glass column, 6 mm outer diameter, filled with Porapack QS.

Radiotracer experiments—Microbial methane production rates (MPR) were measured in the lake-water samples from two principal CH_4 precursors: ^{14}C-labeled sodium bicarbonate ($NaH^{14}CO_3$) and ^{14}C-labeled sodium acetate ($Na^{14}CH_3COO$). Methane oxidation rates (MOR) were determined using ^{14}C-methane (Kuivila et al. 1989). All radioisotopes were purchased from the Isotope Supply Company.

Duplicates of the lake-water samples were collected in 20-mL glass tubes closed with butyl rubber stopper and aluminum crimp seals (Bellco Glass). Sediment samples were taken in 13-mL cut-end Hungate-type glass tubes closed with butyl rubber plungers at one end and rubber septa and screw caps at the opposite side.

One hundred μL of sterile ultrapure water containing either 1.85 MBq of ^{14}C-bicarbonate or 0.37 MBq 2-^{14}C acetate (for the rates of methanogenesis) or 0.37 MBq of ^{14}C-methane (for methane oxidation rates) was introduced to closed samples by injection through the septa with a needled gas-tight Hamilton syringe. After incubation in the dark at in situ temperatures (+4°C) for 24–72 h, samples were fixed by injecting 1 mL of 2 mol L^{-1} NaOH into tubes. Samples were transported and stored in a refrigerator for 5 months until further laboratory processing.

In the laboratory, the $^{14}CH_4$ product of methanogenesis was removed from samples by a stripping and trapping technique. Briefly, ambient air flowing at 100 mL min^{-1} was passed through sample tubes into quartz tubes filled with Ni oxide-covered silica granules, which were contained in an 850°C oven. $^{14}CH_4$ was combusted and the resulting $^{14}CO_2$ was collected in two serial traps, each filled with 20 mL of toluene-based scintillation cocktail with 20% (v/v) phenethylamine. Activity of the $^{14}CO_2$ product was measured by liquid scintillation counting.

Methane oxidation activity was analyzed by placing samples in double-neck 100-mL glass flasks, purging with methane (100 mL min^{-1}) for 1 h to remove $^{14}CH_4$, acidifying with 2 mol L^{-1} H_2SO_4 to pH < 1 to convert fixed ^{14}C-carbonate into $^{14}CO_2$, and subsequently removing liberated $^{14}CO_2$ by nitrogen stripping and trapping in two trap serial vials filled with 20 mL of the same scintillation cocktail.

For determining the rate of sulfate reduction (SRR), 13-mL glass tubes (Hungate type) were filled with lake water and sealed. Each tube was spiked with 100 μL of degassed and sterile water containing 1.85 MBq $Na_2{}^{35}SO_4$, and thereafter incubated in the dark at in situ temperatures. After incubation, samples were mixed with 10 mL of 25% Zn acetate solution in 50-mL plastic centrifuge tubes (to halt microbial processes and protect H_2S from oxidation) and were kept frozen until laboratory analysis. In the laboratory, samples were transferred to the flasks of a distillation device, acidified with 2 mol L^{-1} H_2SO_4 to pH < 1, and stripped with ultrapure N_2. Evolved H_2S was precipitated as ZnS in a bubbling trap with 5 mL of 5% Zn-acetate solution. One hundred μL of 2 mol L^{-1} nonradioactive ZnS suspension was added before distillation to every sample as a carrier. For radioactivity measurements, 2 mL of homogenized ZnS suspension from the traps was mixed with 15 mL of Unisove 100 cocktail (Koch Light Lab.) in 20-mL scintillation vials. The radioactivity of all samples was counted on a Beckman Scintillation Counter.

The rates of the microbial processes were calculated using the equation

$$\text{rate} = \alpha \times r \times C/R \times t \qquad (8)$$

where r is the radioactivity (cpm) of the metabolic product ($^{14}CH_4$ for methanogenesis, $^{14}CO_2$ for methane oxidation, and $H_2{}^{35}S$ for sulfate reduction), C is the substrate pool in waters, and R is radioactivity (cpm) of injected substrates (bicarbonate or acetate for methanogenesis, methane for methane oxidation, and sulfate for sulfate reduction); t is sample incubation time; α is the isotope fractionation coefficient: 1.12 for methanogenesis from bicarbonate (Blair et al. 1993), 1.02 for methane oxidation (Alperin et al. 1988), and 1.06 for sulfate reduction (Jørgensen and Fenchel 1974). All the given rates are gross rates.

Stable isotope analyses—Stable carbon isotope analyses (δ^{13}C) were performed on CH_4, CO_2, and DIC, stable hydrogen isotope analyses (δ^2H) were performed on CH_4 and lake water. The preparation of gas samples (CH_4 and CO_2) for isotope analysis was accomplished using a preparative GC

system connected with separation and combustion lines based on the technique of Dumke et al. (1989). Methane was separated from N_2 and other components with a packed column (Haysep Q, 80–100 mesh, 3 m × 6.35 mm) and then quantitatively converted into CO_2 in an oven filled with cupric oxide at a temperature of 900°C. The resulting CO_2 and H_2O were frozen in a trap at −196°C (liquid nitrogen). CO_2 was separated from H_2O by raising the temperature of the trap to −78°C (dry ice/alcohol mixture). The released CO_2 was collected and thereafter analyzed via mass spectrometry for ^{13}C. The remaining H_2O was transferred into a Supremax tube by raising the temperature of the trap (to ca. 30°C), reduced to H_2 gas with zinc at 600°C, and analyzed via mass spectrometry for ^2H composition. The original CO_2 was separated in the same run using another line without an oxidation oven. Hydrogen isotope analyses on lake-water samples were carried out as described by Meyer at al. (2000).

For ^{13}C analysis of DIC, water samples were collected in dark glass bottles and preserved with $HgCl_2$ until later processing in the laboratory. The DIC of these samples was analyzed on-line using a Finnigan gas-bench system. Only in a few cases was DIC precipitated in the field by adding concentrated solution of $Ba(OH)_2 \cdot 8H_2O$ to an aliquot of water samples. The precipitated $BaCO_3$ was then converted to CO_2 for ^{13}C analyses using the standard phosphoric acid method (McCrea 1950).

Suspended matter was collected by gentle vacuum filtration onto precombusted (450°C) glass-fiber filters (Whatman GF/F), which were then dried and wrapped in aluminum foil until analysis. The particulate organic carbon (POC) content was determined on the powdered GF/F filters using a Leco CHNS-932 determinator.

The stable carbon and hydrogen isotope measurements were carried out on Finnigan MAT isotope ratio mass spectrometers. The isotope ratios are expressed in the usual delta (δ) notation:

$$\delta X\ (‰) = [(R_{\mathrm{sample}}/R_{\mathrm{standard}}) - 1] \times 10^3 \qquad (9)$$

where X is ^{13}C or ^2H and R is $^{13}C/^{12}C$ or $^2H/^1H$. The values are referred to the international standards Vienna Pee Dee Belemnite for carbon and Vienna Standard Mean Ocean Water for hydrogen. Reproducibility of isotope analyses including all preparation steps was approximately ±0.2‰ for carbon and better than ±5‰ for hydrogen.

Results and discussion

Site description—Lake Untersee, the largest freshwater lake (surface area 11.4 km^2) in the interior of East Antarctica (Hermichen et al. 1985), lies in the Otto-von-Grubergebirge of central Dronning Maud Land (71°21′S, 13°25′E) at 563 m above sea level (Fig. 1). The lake, which has a maximum depth of 167 m, is permanently ice covered (Wand and Perlt 1999), drainless, and dammed at its northern end by the Anuchin Glacier (Fig. 2). Lake Untersee receives water from glacial melt mainly supplied by subaquatic melting of the damming glacier. It loses water only by ablation (evaporation) at the surface of the lake ice mainly in summer (Hermichen et al. 1985).

The oxic water body of Lake Untersee is characterized by exceptionally high pH values (9.8–12.1), supersaturation with dissolved oxygen (up to 150%) over the whole depth, Na^+ and SO_4^{2-} as the prevailing ions, very low primary production of phytoplankton (0.11–1.06 mg C m^{-3} d^{-1}, 0.08–0.27 mg chlorophyll *a* m^{-3}), water temperatures mostly below +1°C, high silica contents (up to 14.1 mg Si L^{-1}), and very low concentrations of PO_4-P (≤1 μg L^{-1}) (Kaup et al. 1988; Wand et al. 1997). The salt content (sum of ion concentrations) amounts to approximately 300 mg L^{-1}. The main part of the lake's water body has no significant salinity and temperature gradients. However, in the southern part of the lake, there exists a 500-m-wide and 105-m-deep trough, separated from the remaining lake basin by an underwater threshold with an upper water depth of 30 m (Fig. 2b). This trough has a 20-m-thick anoxic, gas-rich bottom water layer, separated from the overlying oxic water body by a roughly 6-m-thick transition layer (suboxic layer, oxycline). Further anoxic layers in the remaining lake area have not been detected.

Water chemistry and physics—The physical and chemical parameters in the water vary distinctly with depth at the sampling site (Fig. 3; Table 1). Thermoclines, oxyclines, and chemoclines occur at different depth intervals. The pH values are very uniform between 3 and 72 m depth (mean 11.34 ± 0.12) (Fig. 3b). Such unusually high pH values cannot be explained by high concentrations of total dissolved carbonate. They are apparently due to the presence of hydroxyl (OH^-) ions (cf. Cipolli et al. 2004). Moreover, the high SiO_2 concentrations also contribute to the total alkalinity. A sharp drop of the pH occurs between 74 and 75 m depth (from pH 11.0 to 7.6; pH 6.9 at 100 m). In the oxygenated water column, the concentration of dissolved oxygen is rather constant (22.3 ± 1.4 mg L^{-1}), but decreases markedly between 74 and 80 m (oxycline) (Fig. 3a). The water is anoxic below 80 m and smells strongly of hydrogen sulfide. The redox potential (Eh) within the oxygen-rich water is also uniform (+345 ± 10 mV, referred to the normal hydrogen electrode), but drops below ca. 80 m depth to the minimum value of −95 mV at 85 m (Fig. 3d). The local increase of Eh at 74–76 m depth is probably related to the temperature increase (Nernst equation). All the gradients did not change during the austral summers from 1991 to 1995, reflecting a longer persistence of the anoxic region.

The pronounced temperature gradient between 48 and 50 m (thermocline), where no salinity increase occurs (Fig. 3c), is undoubtedly a consequence of the water-density effect (in freshwater systems, water at 4°C has the highest density). The moat forming in warm summers along the sun-exposed southeastern edge of the lake is most probably the source of warmer lakewater that travels laterally along the slope bottom and settles atop the denser (more saline) anoxic layer. The few inflows of glacial meltwater into the lake are too cold (temperature nearly 0°C) and very low in salinity (conductivity 40–50 μS cm^{-1}, according to our own measurements) and can therefore be ruled out as a source. Moreover, the permanent lake ice cover prevents the warming of the uppermost water layer and maintains its temperature consistently between 0°C and +1°C (*see* Kaup et al. 1988).

The comparably warm water layer below 50 m depth ex-

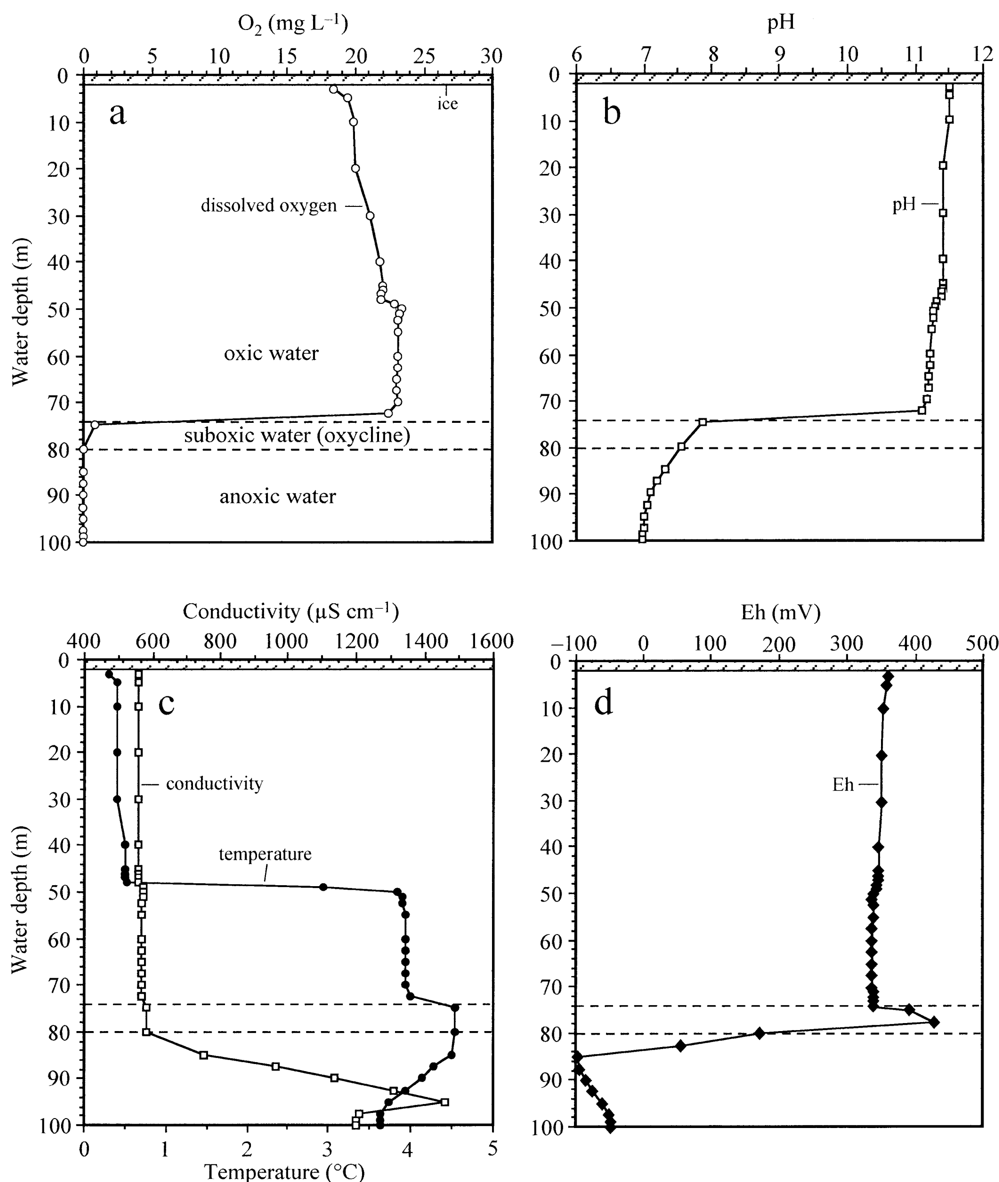


Fig. 3. Depth profiles of (a) dissolved oxygen, (b) pH value, (c) electrical conductivity and temperature, and (d) Eh value at the sampling site in the anoxic trough of Lake Untersee in February 1995, measured in situ.

hibits a temperature peak (+4.6°C) in the 75–85 m depth interval, which can hardly be explained by the above mechanism. It is eye catching that this peak is located in the zone where microbial CH_4 oxidation, sulfate reduction, and possibly sulfide oxidation take place (*see* following sections). This anomaly may be ascribed to energy release in the course of the related biogeochemical reactions. In general, only a fraction of the energy is utilized by microorganisms; the excessive part of energy is available for warming up the water. A comparable temperature phenomenon, but much more pronounced, exists in the particular marine environment of the so-called black holes (Schwabe and Herbert 2004). The temperature peak there is also linked to a microbially active layer of sulfur bacteria. Heating due to microbial activity is, for example, also well known from compost heaps and landfills (e.g., Pirt 1978).

Methane concentration and methane production—Whereas the CH_4 concentration in the oxic part of the water column lies below the detection limit (0.10 μmol L^{-1}) at a depth of 20 m, it peaks near the bottom at 21.8 $\pm$ 1.4 mmol L^{-1} (Table 1; Fig. 4a). This corresponds to 87% saturation under in situ conditions (i.e., under ambient water pressure and temperature) using the solubility data of Yamamoto et al. (1976). Such an extraordinarily high concentration of CH_4 has been very rarely reported for any aquatic environment, including sediments (cf. Heyer 1990), and is quite unusual for oligotrophic systems. Even in Lake Kivu, a gas-rich Central African rift lake, which is considered to be a natural gas deposit (63 $\times$ 10^9 m^3 of CH_4 is accumulated in the deep water), the concentration of CH_4, which is mainly biogenic in origin (Deuser et al. 1973), does not exceed 16.5 mmol L^{-1} (Tietze et al. 1980).

Table 1. Concentrations of selected dissolved components and microbial rates (gross rates) in the water column of Lake Untersee. BLD, below limit of detection; *, incubation time 248 days at +8°C (=net rate); Eh values given relative to the standard hydrogen electrode.

Water depth (m)	pH	Eh (mV)	SO_4 (mg L^{-1})	H_2S (mg L^{-1})	CH_4 (μmol L^{-1})	Methane production via CO_2 reduction (nmol CH_4 L^{-1} d^{-1})	Methane production via acetate fermentation (nmol CH_4 L^{-1} d^{-1})	Methane oxidation (μmol CH_4 L^{-1} d^{-1})	Sulfate reduction (μmol SO_4 L^{-1} d^{-1})
10	11.52	355	163.4		BLD				
20	11.43	352			BLD				
30	11.42	352			0.15				
50	11.31	343	167.7		0.45				
70	11.19	341	165.2		0.65			0.006	
74	10.96	344	161.9		1.15	0.250	0.031	0.009	0.0016
75	7.88	396	160.2		1.49				
76	7.70	408	165.2	3.0	1.85	0.833	0.025	0.010	0.0031
78	7.43	433	162.4		3.50	1.665	0.020	0.052	0.0094
80	7.38	177	159.5		10.2	1.665	0.017	0.020	0.1466
84	7.33	−31	63.8	67.2	2,850	21.647	0.027	0.260	1.8341
85	7.32	−91	68.2		4,900				
86	7.28	−91	73.0		5,300				
88	7.20	−88	48.1		10,200				
90	7.12	−79	25.5		12,350	439.600	0.033	0.31*	0.0025
94	7.03	−56	16.8		15,850				
95	7.01	−55	16.8		17,800				
96	7.01	−51	15.2		17,950	53.285	0.059		0.0012
98	7.00	−44	29.1	0.5	19,700	22.480	0.082		0.0006
99	6.99	−44	14.8		21,800	56.615	0.061		0.0003

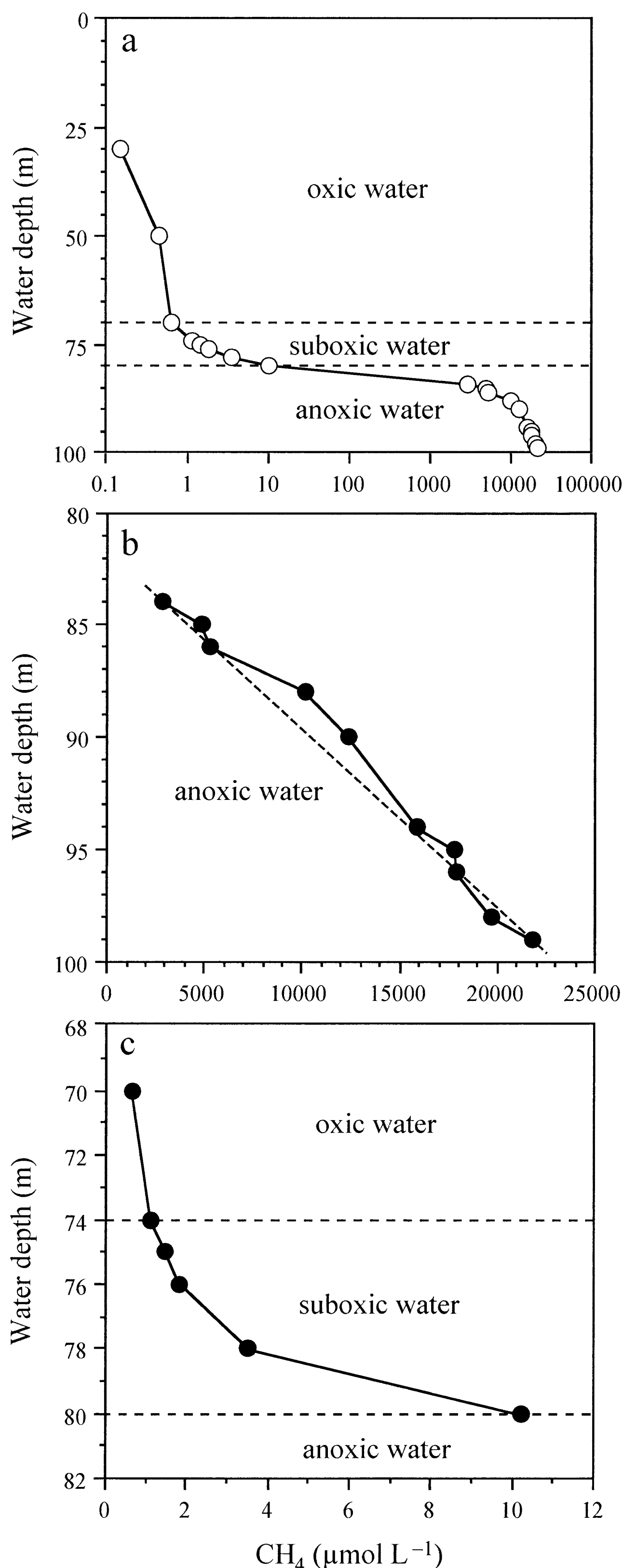


Fig. 4. Distribution of dissolved CH_4 with depth in Lake Untersee, anoxic trough, in February 1995. (a) Variation of the CH_4 concentration (note the logarithmic scale) in the water column. (b) Detailed view of the CH_4 distribution in the anoxic water layer (84 m to bottom). The stippled straight line shows the theoretical decrease in CH_4 concentration caused by diffusion alone. (c) Expanded view of the 70–80-m sampling interval (suboxic layer).

In Antarctic lakes, the only reports on substantial quantities of CH_4 are those of Burton (1980) and Franzmann et al. (1991), who found maximum concentrations of 6.20 and 4.93 mmol L^{-1} in the anoxic hypolimnion of meromictic Ace Lake, Vestfold Hills, respectively. Methane biogeochemistry in the lakes of the Antarctic Dry Valleys, southern Victoria Land, has received little attention. In the (only) detailed study of Smith et al. (1993) on Lake Fryxell in the Taylor Valley, a maximum concentration of 936 μmol CH_4 L^{-1} was measured in the water column (in sediments 1,100 μmol L^{-1}). CH_4 concentrations of up to 60 μmol L^{-1} were determined in the bottom waters of Polyanskii Lake, Bunger Hills, East Antarctica (Galchenko 1994). Although methanogenesis has been found to be a widespread phenomenon in maritime Antarctic lakes (e.g., on Signy Island), the concentration of dissolved CH_4 did not exceed 438 μmol L^{-1} at the sediment–water interface (Ellis-Evans 1984). Therefore, the extraordinarily high concentration of CH_4 in Lake Untersee bottom water was an unexpected finding.

Basically, methane concentration in the water column of Lake Untersee increases in the suboxic layer into anoxic layer and reaches a maximum at the sediment interface (Fig. 4). The shape of the profile suggests CH_4 production emanating from sediment, as well as consumption above ~80 m, because diffusion alone would generate a straight line profile. In the depth interval 70–80 m (suboxic layer), the concentration of CH_4 versus depth increases from 0.65 mmol L^{-1} (3.5% of saturation) to 10.2 mmol L^{-1} (50% of saturation) (Fig. 4c). The concentration pattern has a concave shape, indicating net CH_4 consumption (oxidation) (e.g., Barnes and Goldberg 1976). Below 84 m, the CH_4 concentration gradient is nearly linear (Fig. 4b). Linear CH_4 profiles in sedimentary and aquatic environments are typically caused by diffusion (Fick's Law) (e.g., Weimer and Lee 1973). The CH_4 profile in environments exhibiting CH_4 production has a convex shape (Barnes and Goldberg 1976) or shows a distinct maximum CH_4 concentration several meters above the sediment (Winfrey and Zeikus 1979). Despite a measurable CH_4 production below 80 m, i.e., within the anoxic water layer (*see* Fig. 5), the straight line CH_4 profile is, however, not significantly changed in this depth range.

An attempt to measure the CH_4 concentration in the surficial lake sediments at the anoxic site was made. Unfortunately, the uppermost 0.3 m of sediment cores from the anoxic trough degassed intensively upon recovery, preventing detailed and accurate CH_4 profiling. However, the CH_4 bulk concentration for this horizon could be roughly estimated by head-space analysis to be at least 20 mmol L^{-1}_{sed}. This points to surficial sediment as the major source for CH_4 observed in the water and diffusion as the main process controlling the CH_4 distribution within the anoxic water layer (large concentration gradient).

The highest rate of methanogenesis in the water column was 0.44 μmol L^{-1} d^{-1} at a depth of 90 m. This activity is similar to that in other Antarctic lakes (Vestfold Hills, Bun-

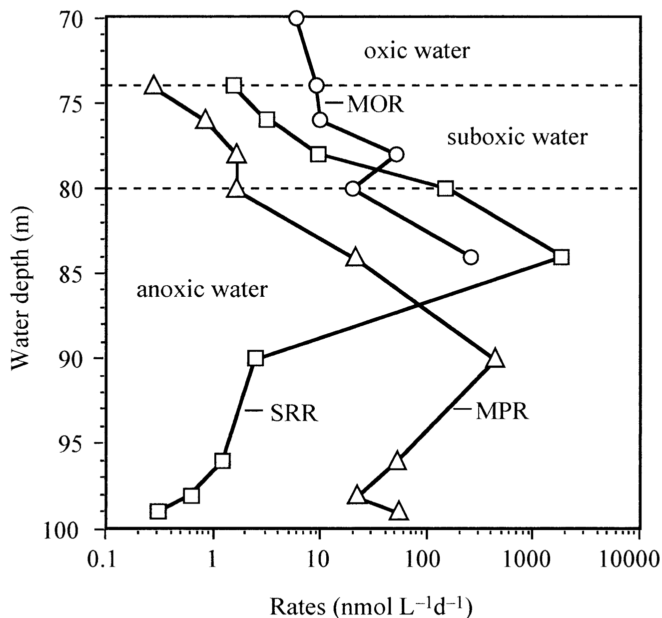


Fig. 5. Rates of methane production (MPR), methane oxidation (MOR), and sulfate reduction (SRR) in the water column of Lake Untersee as a function of water depth (sampling date: February 1995), determined by radioisotope (^{14}C, ^{35}S) tracer techniques. All measured rates peak within the anoxic zone. MPR represents the sum of production via H_2/CO_2 reduction and from acetate fermentation. Methane is consumed by both aerobic and anaerobic oxidation, the anaerobic MOR being higher than the aerobic MOR.

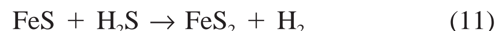
ger Hills, Signy Island) (e.g., Franzmann et al. 1991; Smith et al. 1993; Galchenko 1994). The highest MPR reported for Antarctic lake water was 2.5 μmol CH_4 L^{-1} d^{-1} in Ace Lake, Vestfold Hills (Franzmann et al. 1991). The maximal MPR in anoxic waters of temperate zone lakes is generally lower, e.g., 0.72–1.44 μmol L^{-1} d^{-1} in meromictic Knaack Lake (Winfrey and Zeikus 1979) and only 0.012 μmol L^{-1} d^{-1} in Big Soda Lake (Iversen et al. 1987).

Our radiotracer experiments with ^{14}C (Table 1) reveal that CH_4 is mainly produced via H_2/CO_2 reduction (89–100% of the total CH_4) in Lake Untersee. Acetoclastic methanogenesis is quantitatively unimportant. The CO_2-reduction pathway is rarely observed in freshwater environments and more typically observed in marine sediments (Crill and Martens 1986). It does, however, seem to be a feature of polar freshwaters (Ellis-Evans 1984; Galchenko 1994). This pathway is probably favored in the anoxic fresh waters of Lake Untersee because of high DIC concentrations (Table 2). Freshwater ecosystems commonly have much lower DIC concentrations, mostly below 2 mmol L^{-1} (Bade et al. 2004). The diminished availability of labile organic substrates (acetate: 5.5–11.0 μmol L^{-1}) (Table 3) obviously limits the activity of fermentative methanogens as shown, e.g., by Kuivila et al. (1989). Acetate threshold concentrations for acetate-based methanogenesis reported in the literature vary between 7 and 1,180 μmol L^{-1} (cit. He and Sandford 2004). According to Lovley and Klug (1986), an acetate concentration of at least 21.5 μmol L^{-1} is needed for net growth of methanogens in freshwater sediments. The half-saturation constant for acetate-based methanogenesis is usually 2–3 times higher than the threshold value. Moreover, an inhibition of acetoclastic methanogenesis by hydrogen sulfide (*see* Heyer 1990) has to be taken into account.

Molecular hydrogen necessary for CO_2 reduction is mainly produced as an intermediate during microbial degradation of organic substances by fermenting and syntrophic microorganisms, e.g., by the overall reaction

$$\text{glucose} + 2H_2O \rightarrow 2\text{acetate} + 2CO_2 + 4H_2 \quad (10)$$

or by chemical reactions such as



$$FeS + H_2S \rightarrow FeS_2 + H_2 \quad (11)$$

In view of the unusually high FeS_2 content (up to 4.8 weight percent) determined in the surface lake sediments (Schwab 1998), reaction (11) could be plausible.

Our finding of the dominance of the CO_2-reduction pathway is in contradiction to the assumption that low temperatures (+4°C) apparently limit methanogenesis from H_2/CO_2 (Schulz and Conrad 1996; Nozhevnikova et al. 1997). Methanogenesis is controlled by additional factors such as substrate availability, pH, redox potential, and competitive electron acceptors (SO_4^{2-}, NO^{3-}, Fe^{3+}). Regarding temperature limitation, Schulz et al. (1997) pointed out that hydrogenotrophic methanogens are not influenced by the low temperature itself but rather by the insufficient supply of hydrogen by H_2-producing syntrophs at low temperature. Additional evidence of hydrogenotrophic methanogenesis at low temperature has been documented in tundra soils (Samarkin et al. 1999) and Antarctic lake sediments, where this pathway also dominated ($\geq$80%) (e.g., Ellis-Evans 1984; Galchenko 1994; Mountfort et al. 1999). A psychrophilic hydrogenotrohic species of methanogen has been described by Franzmann et al. (1997). Mountfort et al. (1999) demonstrated in an Antarctic meltwater pond that hydrogenotrophic methanogenesis is enhanced by temperature and H_2 supply. This group of methanogens is obviously mainly substrate limited (Nozhevnikova et al. 2003). Thus, we conclude here that CH_4 production in the water column of Lake Untersee is most likely controlled by temperature and substrate (DIC and H_2) availability.

Further evidence of methanogenesis from CO_2 and H_2 is provided by the apparent isotope fractionation factor α calculated from the measured stable carbon isotope composition of coexisting CH_4 and CO_2 in Lake Untersee water. Marine sediments, where the CO_2 reduction pathway is generally preferred, are characterized by $\alpha_C > 1.05$ and $\alpha_H < 1.25$ (average 1.22), freshwater sediments (fermentation pathway preferred) by $\alpha_C < 1.05$ and $\alpha_H > 1.39$ (average 1.43) (Whiticar et al. 1986). The mean α values within the CH_4 production zone of Lake Untersee water below 90 m depth ($\delta^2H_{H_2O} = -282‰$, our own measurement) are

$$\alpha_C = 1.073 \pm 0.002 \quad \text{and}$$

$$\alpha_H = 1.20 \pm 0.02 \qquad (n = 4)$$

clearly pointing to values typical of the marine environment, in which the CO_2 reduction pathway dominates, albeit the measured $\delta^{13}C$ values of $\sim -50‰$ (*see* Table 2) are relatively high for CH_4 derived from this pathway (generally between $-110‰$ and $-60‰$ according to Whiticar et al. 1986). This

Table 2. Vertical distribution of dissolved and suspended matter in Lake Untersee water and stable isotope characteristics of some components. DIC, dissolved inorganic carbon; DOC, dissolved organic carbon; POC, particulate organic carbon; VPDB, Vienna Pee Dee Belemnite; VSMOW, Vienna Standard Mean Ocean Water; for definition of α, see Eqs. (6) and (7).

Depth (m)	DIC (mg L^{-1})	DOC (mg L^{-1})	POC (mg L^{-1})	δ^{13}C-DIC (‰) VPDB	$CH_4/(CH_4+CO_2)$ (volume ratio)	δ^{13}C-CH_4 (‰) VPDB	δ^{2}H-CH_4 (‰) VSMOW	δ^{13}C-CO_2 (‰) VPDB	α_C	α_H
Oxic layer										
10	6	0.58	0.007							
30			0.008	4.29						
50	7	0.66	0.009							
70	3	0.61	0.010							
71	8	0.74	0.010							
72	6	0.51	0.009							
73	2	0.78	0.013							
Suboxic layer (oxycline)										
74	23	0.97	0.016	4.41						
75	28	1.09	0.030	4.81						
76	24	1.00	0.019	5.36						
77	25	1.07	0.025	4.35	0.08	−31.0	−164	−2.0	1.030	0.859
78	25	0.95	0.018	4.42						
79	23	0.97	0.006	5.54						
Anoxic layer										
80	25	0.98	0.012					−6.8		
82	45	1.15	0.026							
84	227	3.28	0.030	15.50	0.79	−51.5	−317	+8.9	1.064	1.051
86	228	3.52	0.020	14.40						
88	332	4.52		18.32						
90	439	6.23	0.021	25.94	0.92	−50.9	−402	+18.0	1.073	1.201
92	474	7.02		26.80						
94	535	7.26	0.035	27.85						
96	574	7.97	0.231	27.58	0.89	−48.1	−393	+18.2	1.070	1.183
98	560	8.71	0.483	28.37	0.89	−49.0	−409	+21.2	1.074	1.215
99	679	13.50	1.271	30.86	0.89	−49.1	−392	+21.6	1.074	1.181
100	711	15.63	1.788	29.02						

Table 3. Acetate concentrations in Lake Untersee water and sediment (anoxic trough).

	Acetate (mg L^{-1})	Acetate (μmol L^{-1})
Water depth (m)		
75	0.39	6.49
80	0.33	5.50
85	0.62	10.32
90	0.37	6.16
95	0.63	10.49
99	0.66	10.99
Sediment depth (cm)		
0–5	14.56	240
5–10	11.60	190
10–15	12.24	200
20–25	15.00	250
25–30	14.24	240
30–35	17.28	290
35–40	18.40	310
40–45	12.80	210
45–50	11.04	180

increased $\delta^{13}C_{CH_4}$ value may be ascribed to the isotopically very heavy nature of the source DIC ($\delta^{13}C_{DIC}$ ranging between +14.40‰ and +30.86‰ in the anoxic water) (Table 2).

As Waldron et al. (1999) concluded from field and laboratory data, the $\delta^2H_{CH_4}$ in sulfate-poor, shallow freshwater environments is directly linked to the hydrogen isotope composition of the environmental water rather than to the preferred methanogenic pathway, as proposed, e.g., by Whiticar et al. (1986) and Sugimoto and Wada (1995). This conclusion is confirmed by our data. The measured stable hydrogen isotope composition of CH_4 in Lake Untersee waters ($\delta^2H_{CH_4}$: ~−400‰; $\delta^2H_{H_2O}$: −282‰) is not clearly indicative of the preferred pathway when using the relationship derived by Sugimoto and Wada (1995) on the basis of laboratory experiments or by using the discrimination diagrams constructed from field observations (fig. 10 in Whiticar 1999). Based on the equation given by Sugimoto and Wada (1995), the calculation of $\delta^2H_{CH_4}$ produced by CO_2 reduction yields an unrealistic value of −510‰ ± 25‰. The use of the relationships of Waldron et al. (1999) yield somewhat more reliable $\delta^2H_{CH_4}$ (−474‰ and −446‰) for Lake Untersee methane. These authors emphasize, however, that for other environments, different relationships may be valid. This shows that the noncritical use of such diagrams or relationships may lead to misinterpretations with methanogenic pathway and environment identifications.

Measurements of the MPR in the surficial sediment of Lake Untersee yield a maximum rate (total MPR = 0.88 μmol CH_4 dm^{-3} d^{-1}) twice that measured in the water column; CO_2/H_2 reduction is also the major methanogenic pathway in the sediments (Table 4), even though the acetate concentration in the sediments (180–310 μmol L^{-1}) (Table 3) cannot be a limiting factor. The MPRs in sediments of temperate freshwater lakes are often significantly higher compared with those in Antarctic lakes (Winfrey and Zeikus 1979; Heyer 1990; Dzyuban 2002). The highest rates (in Baltic lakes up to 950 μmol L^{-1} d^{-1}) were measured in eutrophic and hypereutrophic lakes (Dzyuban 2002). Besides substrate availability, the MPR is clearly affected by the temperature. The relatively low MPRs in Antarctic lakes sediments (maximum 80 μmol dm^{-3} d^{-1} below microbial mats as determined Mountfort et al. 1999) are therefore certainly due to the fact that temperature is a significant factor limiting methanogenesis in permanently cold environments (e.g., Zeikus and Winfrey 1976; Ellis-Evans 1984; Franzmann et al. 1991). For instance, Mountfort et al. (1999) found in an Antarctic low-salinity freshwater pond that the maximal MPR at in situ temperatures (2–4°C) were fourfold to fivefold lower than rates measured at 20°C. They also demonstrated that the addition of nitrate, sulfate, and chloride at high levels (>10 mmol L^{-1}, i.e., high salinity) can inhibit methanogenesis.

Methane oxidation—As already mentioned, the CH_4 distribution pattern in Lake Untersee waters has been affected by consumption of CH_4. There was clear evidence of aerobic microbial CH_4 oxidation within the oxic (to suboxic) water of Lake Untersee from radiotracer experiments (Table 1). The concave CH_4 profile itself in the 70–80-m interval, i.e., mainly within the suboxic layer (Fig. 4c), points undoubtedly to oxidation of CH_4. Such concentration profiles are

Table 4. Methane production in sediments from Lake Untersee (anoxic trough) and relative contribution (%) of the methanogenic pathways. ND, not determined.

Sediment depth (cm)	Acetate (mmol L^{-1})	Methane production from H_2/CO_2 (nmol dm^{-3} d^{-1})	Methane production from acetate (nmol dm^{-3} d^{-1})	Methane production total (nmol dm^{-3} d^{-1})	% methane from H_2/CO_2	% methane from acetate
0–5	0.24	382.98	5.00	387.98	98.7	1.3
5–10	0.19	516.20	15.82	532.02	97.0	3.0
10–15	0.20	874.20	6.66	880.86	99.2	0.8
15–20	ND	790.95	12.49	803.43	98.4	1.6
20–25	0.25	732.67	19.15	751.82	97.5	2.5
25–30	0.24	532.85	9.16	542.01	98.3	1.7
30–35	0.29	358.01	6.66	364.67	98.2	1.8
35–40	0.31	407.96	23.31	431.27	94.6	5.4
40–45	0.21	258.10	19.15	277.25	93.1	6.9

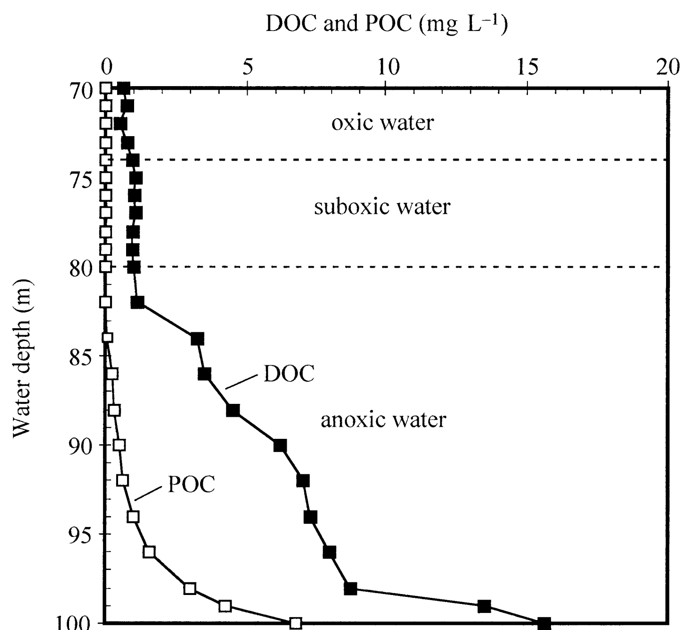


Fig. 6. Vertical distribution of dissolved and particulate organic carbon (DOC and POC, respectively) in the anoxic trough of Lake Untersee.

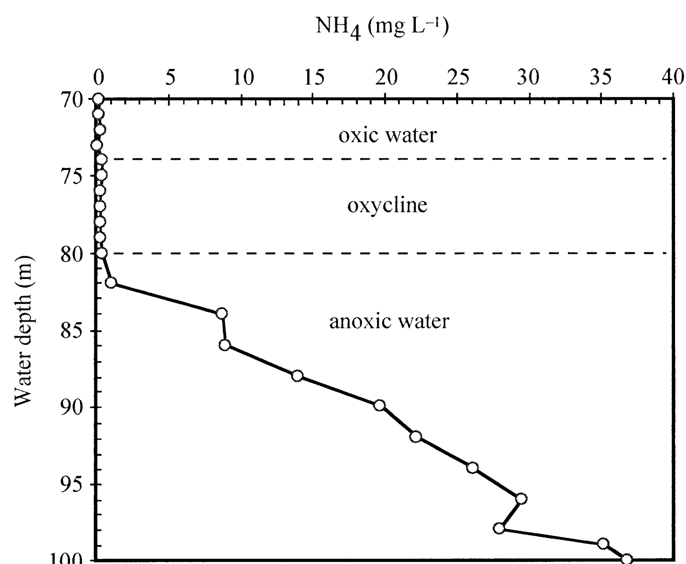


Fig. 7. Depth profile of ammonium in the water column of Lake Untersee (anoxic site).

characteristic of the interface between oxic and anoxic layers in methanogenic environments (e.g., Whiticar 1999). Both the stable isotope composition of CH_4 (δ^{13}C: −31.0‰; δ^2H: −164‰) and the isotope fractionation between CH_4 and CO_2 (α_c = 1.030) from 77-m depth (suboxic layer), where the CH_4 concentration profile is concave, support the occurrence of aerobic CH_4 oxidation (Coleman et al. 1981; Whiticar 1999).

However, there is further evidence from radiotracer experiments of anaerobic CH_4 oxidation between 74- and 84-m depth (Table 1). The MOR from greater water depths could not be determined in short time course experiments because of strong dilution of $^{14}CH_4$ due to high concentrations of unlabeled CH_4 in the water samples. Hence, future direct measurements of anaerobic MOR using radiolabeled $^{14}CH_4$ of high specific activity are necessary.

Based on CH_4 distribution alone methane oxidation in the anoxic layer cannot not be revealed since methanogenesis overprints the effect of CH_4 oxidation (MPR at 84 m is by a factor of ca. 80 higher than anaerobic MOR!). Anaerobic methane oxidation is not uncommon for anaerobic water columns (Reeburgh and Heggie 1977; Iversen et al. 1987; Joye et al. 1999). Anaerobic MOR for Antarctic lakes (sediment and water) have hitherto been measured only in lakes of the Bunger Hills (Galchenko 1994). In Lake Fryxell, Dry Valleys of southern Victoria Land, anaerobic CH_4 consumption in the water column was suggested based solely on the concave CH_4 concentration profile (Smith et al. 1993).

It is worth noting that anaerobic methane oxidation in Lake Untersee was detected in the zone of maximal sulfate reduction (Fig. 5) (sulfate reduction in Lake Untersee will be described elsewhere in more detail). This is in agreement with the observation that peak rates of anaerobic methane oxidation generally occur near the sulfate-methane transition zone (e.g., Devol and Ahmed 1981). Field and laboratory studies suggested that a consortium of methanogenic and sulfate reducing microorganisms is responsible for this process (Hoehler et al. 1994).

Because sulfate reduction in Lake Untersee will be described in more detail elsewhere, it is only pointed out here that the maximal SRR in Lake Untersee waters (1.83 μmol SO_4 L^{-1} d^{-1}) is higher than that in other Antarctic lakes: Burton Lake, Vestfold Hills (0.59 μmol L^{-1} d^{-1}; Franzmann et al. 1988) and in the lakes of Bunger Hills (0.16 μmol L^{-1} d^{-1}; Galchenko 1994), but similar to that in monimolimnion of Big Soda Lake, Nevada (0.9–3.0 μmol L^{-1} d^{-1}; Smith and Oremland 1987).

Anaerobic oxidation of methane at 84 m is not seen in its δ^{13}C value, but seems to be indicated by $\delta^2H_{CH_4}$, $\delta^{13}C_{CO_2}$, $CH_4/(CH_4 + CO_2)$, and α_c (=1.064). The CH_4 concentration at this depth is too high (2.85 mmol L^{-1}) and the MOR rather slow, masking the influence of oxidation on $\delta^2H_{CH_4}$. On the other hand, the CO_2 fraction is relatively low, so that the mixing with isotopically light CO_2 formed by anaerobic oxidation (Alperin et al. 1988) is more clearly reflected.

Origin of the high CH_4 concentrations—The question arises on the origin of the CH_4 anomaly. Surficial lake sediments in the anoxic trough are clearly the main source of CH_4 dissolved in the anoxic water. For the microbiological processes observed in the anoxic waters, organic and inorganic carbon sources are needed. Although the primary productivity in the top 50 m of the water column is extremely low (Kaup et al. 1988), it can account for the DOC and POC enrichment observed in the depth (Fig. 6). Because there is no flushing of the water at depth, organic carbon has been accumulating there for long periods (decades, if not centuries). As a consequence, surficial lake sediments (up to 30-cm depth) in the anoxic trough contain between 1.5 and 4.5 wt.% C_{org} (unpubl. data).

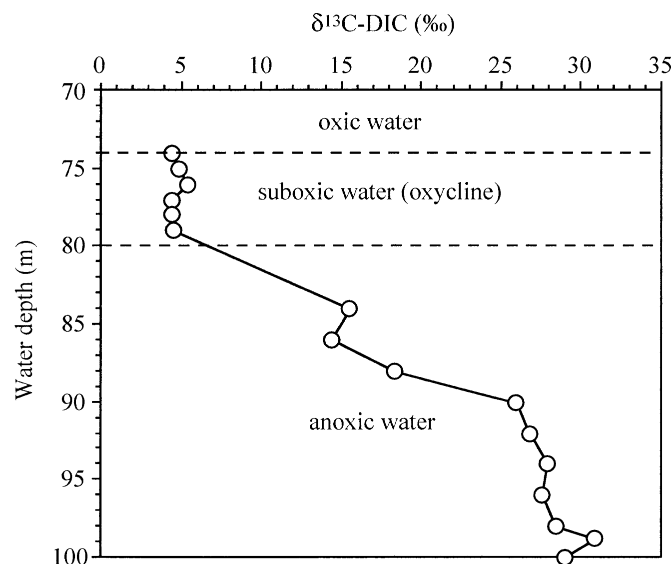


Fig. 8. Variation of $\delta^{13}C_{DIC}$ in the water column of Lake Untersee (anoxic site).

The increase of DIC with depth may be due to upward migration from the sediment into the water column. Diffusive upward flux from the sediment is obviously the predominant mechanism for CH_4 accumulation in the water column, which is underlined by the almost linear shape of the CH_4 profile. This holds also for other constituents, such as dissolved NH_4 (Fig. 7) (positive linear correlations NH_4/depth, and CH_4/NH_4, with $r^2 = 0.98$, in the 84–99-m interval). The accumulation of substantial quantities of CH_4 in the water column is favored by very stable waters. Wind-driven mixing is prevented by the permanent ice cover existing for hundreds of years (Wand and Perlt 1999). Moreover, both low temperature and low salt content increase the solubility of methane in water.

In summary, oligotrophic Lake Untersee is an exceptional Antarctic freshwater lake exhibiting significant microbial activities in its anoxic part of the water column. It is one of the very few documented active methanogenic environments on continental Antarctica. The concurrence of methanogenesis, methane oxidation, and sulfate reduction measured in Lake Untersee is rather rarely observed in water-column environments. Intense CH_4 production (maximum 0.88 μmol dm^{-3} d^{-1}) in the underlying C_{org}-rich sediments, upward diffusion, additional CH_4 production in the water column (maximum 0.44 μmol L^{-1} d^{-1}), and a stable amictic water column (due to the perennial ice cover) create an impressive biogenic CH_4 enrichment in the lake water. Gross production of CH_4 exceeds oxidation, and molecular diffusion is the only removal term. The measured methane production rates are comparable with those from other Antarctic lakes. Their magnitude is site specific and mainly controlled by substrate supply. The stable isotopic signature of DIC both in the pore water (+28.0‰ ± 1.1‰, $n = 7$) and in the anoxic lake water (Fig. 8) are typically caused by methanogenesis.

Our studies show, furthermore, that H_2/CO_2 reduction may sometimes be the major pathway of methanogenesis in low-sulfate freshwater environments even at low temperatures. This is consistent with the assumption of Ellis-Evans (1984) that this pathway is probably more important in Antarctic lakes than hitherto assumed. The preference of the H_2/CO_2 reduction pathway in the Antarctic ecosystems so far studied is probably due to differential sensitivities of microbial populations to temperature and temperature-dependent substrate affinities at low temperatures (Mountfort et al. 1999). The stable isotope analyses of the dissolved methane demonstrate that the discrimination of the major methanogenic pathways using $\delta^{13}C$-CH_4/$\delta^{2}H$-CH_4 plots alone may be sometimes problematic and that the isotope fractionations derived from laboratory experiments should be applied with caution to natural conditions. ^{14}C labeling or other techniques, such as inhibition and stimulation experiments (*see* Conrad 2005), appear to be more convenient tools for tracking the principal methanogenic pathway.

Our findings demonstrate the activity of cold-adapted microbial communities in permanently cold lacustrine environments and demonstrate the importance of biogeochemical processes in organic-matter mineralization also in such harsh aquatic environments as Antarctic lakes. More research is needed to evaluate, in particular, the role of H_2 in the complex process of organic-matter mineralization in the Antarctic ecosystems. Our results may have some relevance for the production of methane gas hydrates in tundra soils and in the deep ocean.

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