CARBON ISOTOPIC COMPOSITION OF EARLY-DIAGENETIC METHANE: VARIATIONS WITH SEDIMENTS DEPTH

Mariusz Orion Jędrysek[1], Stanisław Hałas[2], Tomasz Pieńkos[2]

[1]Laboratory of Isotope Geology and Biogeochemistry, Institute of Geological Sciences, University of Wrocław, 50-205 Wrocław, Poland.
[2]Mass Spectrometry Laboratory, M. Curie-Skłodowska University. 20-031 Lublin, Poland.

ABSTRACT

We demonstrate the annual cycle of methane in fresh sediments of two lakes – Moszne (E Poland) and Skrzynka (W Poland). The vertical gradient in $\delta^{13}C(CH_4)$ values varied widely from about -4.5‰/(-1 m) in late summer 1993 to about +2.5‰/(-1 m) in late winter, in the uppermost sediment profiles of about 3-meters in length. These vertical variations apparently are not due to oxidation or temperature changes, but rather to the higher gradient of the downward decrease of production rates via the acetic acid fermentation pathway rather than via the CO_2-H_2 pathway. The production of methane and $\delta^{13}C(CH_4)$ values are the highest during summer while the lowest during winter, reflected especially during surface sampling. The downward gradient of $\delta^{13}C$ in winter, late autumn and, at greater depths, in late summer results from isotope enrichment of the residual pool of precursors of methane, predominantly CO_2.

Keywords: carbon, isotope, methane, lake, sediment, diagenesis, greenhouse effect, isotope budget

1. INTRODUCTION

Methane production is an important part of the global carbon cycling (e.g. Craig and Chou 1982, Khail and Rasmussen 1983, Steele et al. 1987) and methanogenic pathways are determined by coexisting organic matter, water, and bacterial activity. The following factors have been proved or proposed to affect methanogenesis and methane flux from natural wetlands: (1) salinity (De Laune et al. 1983), (2) fertilization of paddy soil (Wada 1990), (3) soil redox potential (Cicerone et al. 1983, Svenson and Rossewall 1984), (4) nutrient and organic content of surface soils and their thickness (Harriss and Sebacher 1981). (5) substrate and volatile end-product concentration (De Laune et al. 1986), (6) plant type and its physiological state (ventilation rate, age, height; Dacey and Klug 1979, Dacey 1981, Cicerone et al. 1983, Sebacher et al. 1985), and (7) primary production (Whiting and Chanton 1993).

In addition many external factors like (1) wind speed (Sebacher et al. 1983), (2) atmospheric pressure (Matson and Likens 1990), (3) moisture content or water level (Harriss et al. 1982, Svenson and Rosswall 1984), and (4) temperature (Baker-Blocker et al. 1977, Svenson and Rosswall 1984, Sebacher et al. 1986) may control the methane flux from natural wetlands. In this paper we report seasonal variations in vertical profiles in concentration and carbon isotopic composition of methane.

Basing on the thermodynamics, an implicit assumption could be apparently made that carbon isotope fractionation factor between the methane precursors and methane gas, depends on temperature. Logically, at the lower temperature of the lake environment, the fractionation factor could be expected to be larger. Other factors controlling isotope composition of methane are isotope ratio in substrates (methane precursors) and methane consumption by microbial oxidation. In natural freshwater systems, the fermentation of acetate, $CH_3COOH \rightarrow CH_4 + CO_2$ (Barker 1936), and the reduction of carbon dioxide, $CO_2 + 4H_2 \rightarrow CH_4 + H_2O$ (Takai 1970), are the main methanogenic pathways. Based upon freshwater paddy soil incubation experiments, the endmember $\delta^{13}C$ value of methane produced from acetate dissimilation is -36‰ and the $\delta^{13}C$ value of CH_4, from CO_2/H_2 was estimated to be -77 to -60‰ (Sugimoto and Wada 1993). Therefore, changes in the relative ratios of methane formation pathways may lead to temporal and spatial variations of the $\delta^{13}C$ value in CH_4, though an organic matter precursor may show homogeneous carbon isotopic composition.

It has been shown that, in natural conditions, diurnal variations of $\delta^{13}C(CH_4)$ in some cases does but, usually does not correspond to the diurnal variations of water or sediments temperature, but rather corresponds to the abundance and isotope characteristics of the methane precursors with negligible role of methane oxidation (Jędrysek 1995, 1997). Therefore, unknown factor(s) still remain for

natural conditions of freshwater lakes systems, because of lack of information on spatial and temporal variations of methanogenesis in freshwater sediments, particularly in lakes (e.g. Woltemate et al. 1984, Whiticar et al. 1986).

It was found recently that $\delta^{13}C(CH_4)$ values of freshwater methane tends to become more negative with depth of the water column and depth of sediments (Jędrysek et al. 1994, Jędrysek 1997, 2005a). Consequently, one may state that data published in previous works may not firmly represent isotopic composition of total methane emitted from freshwater sediments. Therefore, the goals of the present study are: 1) to obtain new data on CH_4 generated from lakes with respect to spatial and temporal distributions, 2) better understanding of the mechanisms of methanogenesis, 3) to provide new data for isotopic mass balances of carbon cycling in early diagenesis in freshwater systems and greenhouse gases flux.

2. MATERIALS AND METHODS

2.1. STUDY AREA

Methane was sampled in two lakes, one in Eastern another in Western Poland. A lake in Eastern Poland, located ca. 50 km NE from Lublin (5l.4°N, 23.1°E), was selected in this study as the main sampling site. It belongs to the region of the Łęczna–Włodawa Lakeland. The water balance clearly shows that this region is short of water. Mean precipitation, of many years measured, is 560 mm and evaporation is 450 mm. About 110 mm is runoff of which 52% makes the underground runoff. Small permeability and water capacity of Pleistocene deposits underlying the lake and the fact that the area is flat, with a dozen or so meters of maximum relative height, are the reasons that the water level is not deep and the surface is swampy (Wilgat et al. 1991). Lake Moszne is in its final stage of the lake development, it is surrounded by peat-bogs, swamps and marshes. It is a dystrophic, very shallow (max. depth 0.8 m), small (0.17 km^2) lake. No seasonal variations in the water level has been observed. The bottom is very flat and practically free from any macrophytes. All the bank of the lake around is covered by a floating peat-bog. The sediments are extremely soft and composed near exclusively of *in situ* organic matter detritus (C_{org} in the dry sediment is about 95%).

FIG. 1. Map showing the locations of sampled lakes in Poland. Sampling sites are described in: Jędrysek (1995, 1997, 1999).

Occasionally sampling have been carried out also in Lake Skrzynka (52.15°N, 17.0°E, ca. 30 km southward from Poznań, W Poland). Lake Skrzynka is max. 2.0 meters depth and shows near identical limnological and hydrological character as Lake Moszne, but is situated in much less marshy region.

2.2. SAMPLING AND FIELD OBSERVATIONS

In order to reduce the influence of diurnal variations (Jędrysek 1994, 1995) each sampling campaign was carried out between 12:00 and 3:00 PM. Vertical sampling profile was done by sequential agitation of deeper zones of sediment by means of a scaled (with 1 cm accuracy) paddle. The depth of water in the sampling sites was 0.5 m. The visually estimated sampling depth resolution was apparently better than 5 cm. Bubbles were obtained from submerged sediments by agitation, and then trapped by an inverted funnel ~ 20 cm in diameter into a glass bottle filled with gas-free water. The uppermost layer of sediment was agitated first, until all the methane had apparently been released. Then, the next, deeper zone of sediments was stirred, and in each subsequent zone the thickness of the stirred sediments was narrower. This procedure apparently prevented contamination of methane from a given zone by methane from another overlaying zone.

Sampling time of bubble gases was usually not longer than 2 minutes. Some water remained in the bottle. The bottles were sealed with a butyl rubber cap and an aluminum seal, and the samples were immediately treated with $HgCl_2$. Bottles with samples were stored in a refrigerator (3–4°C) in an inverted position. Experiments with analysis repeated at different times for several times proved that the length of the period for which samples were held before they were analyzed did not influence results. Moreover, it was proved previously that samples collected at the same time from the same depth of water column, from the same depth of sediments (0–25 cm) and from reasonably similar sediments, but at

different sampling stations, showed the same $\delta^{13}C$ results within analytical error which was from 0.05 to 0.2‰ (Jędrysek et al. 1994, Jędrysek 1994, 1995). Vertical profiles of temperature in sediments were measured with a precision of 0.1°C, using a 3-meter long thermocouple probe made by "Czaki".

2.3. ANALYTICAL TECHNIQUES

With molecular sieves, a dry-ice-ethanol mixture, and liquid nitrogen, the methane was cryogenically purified under vacuum from other hydrogen and carbon containing gases. Subsequently, the methane, together with hydrogen and carbon-free gases was passed through a copper oxide furnace (850–900°C) twice. The products obtained, H_2O and CO_2, were separated cryogenically. Carbon isotope analyses were made on a modified MI-1305 mass spectrometer with dual inlet (Halas, 1979) and home-made detection systems (Hałas and Skorzyński, 1980). The isotope ratios are expressed as $\delta^{13}C$ values relative to the PDB standard, using a mass spectrometric comparison of working CO_2 gas with CO_2 prepared from NBS 19 and NBS 22 standards. The internal precision obtained was 0.05‰. The reproducibility of isotope preparation was from ±0.05 to ±0.2%). The chemical composition of sample gases was analyzed by TCD gas chromatography ("Elwro chromatograph S04") and the content of organic matter (%C_{org}) was analyzed by Differential Thermal Analysis and Thermogravimetry ("Derivatograph 1500D").

3. RESULTS

All data have been presented in figures. Symbols, in each figure representing the same lake, corresponds to the same profile.

Since temperature is the factor controlling isotope effects, temperature measurements in the sediments profiles have been carried out. In the spring of 1993, profiles of temperature (Fig. 2a) in Lake Moszne tend to decrease to sediment depth of ~0.8 m, and then increase at the greater depths. In the early and late summer 1993, the temperature of sediments decreases downwards. Some extra temperature profiles, which are not accompanied by gas sampling, are shown in Fig. 2a. However, in very late summer, in the Lake Skrzynka sediments, the highest temperature has been found at the depth of about 0.7 m (Fig. 2b). In the autumn 1993 the temperature tended to increase to sediment depth of ~1.3 m, and then decreased at greater depth. In the winter 1994 the temperature increased downwards. Sampling in 92.09.27 (Lake Moszne) and 93.05.23 (Lake Skrzynka) have not been associated with temperature measurements.
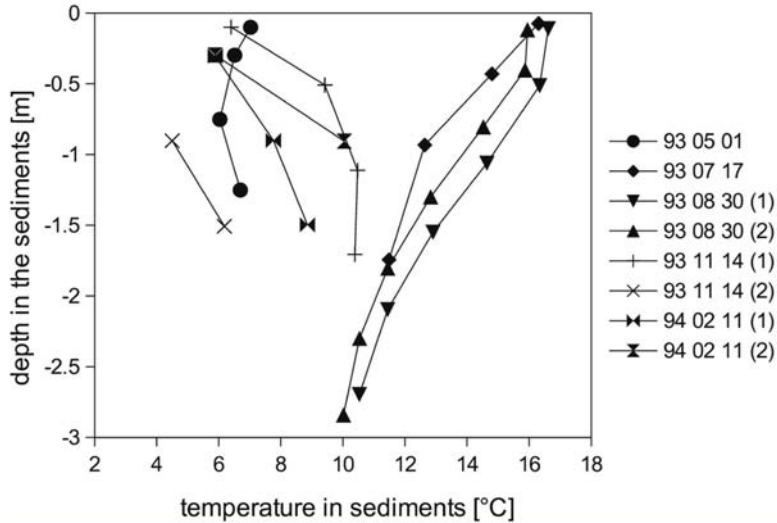
FIG. 2a. Temperature variations in vertical profiles in the Lake Moszne sediments (E Poland), each point corresponds to the gas sampling interval, temperature was measured in 10 cm of vertical intervals. All the symbols in each figure representing Lake Moszne refer to the same profile. Some additional temperature profiles has been shown here, but they are not represented by samples.
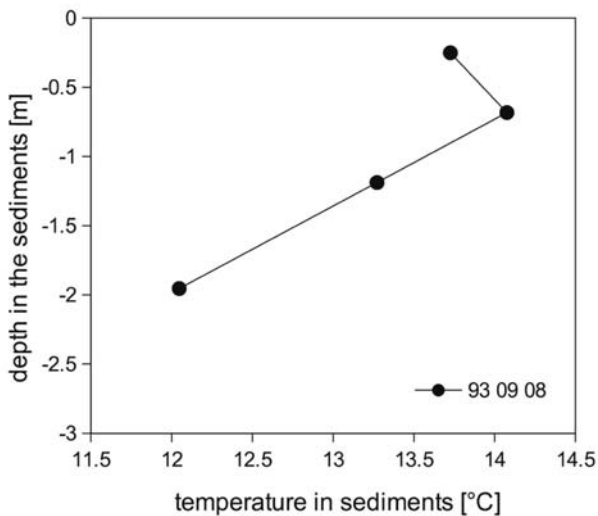

FIG. 2b. Temperature variations in vertical profiles in the Lake Skrzynka sediments (W Poland), each point corresponds to the gas sampling interval, temperature has been measured in 10 cm intervals. All symbols in each figure representing Lake Skrzynka corresponds to the same profile.
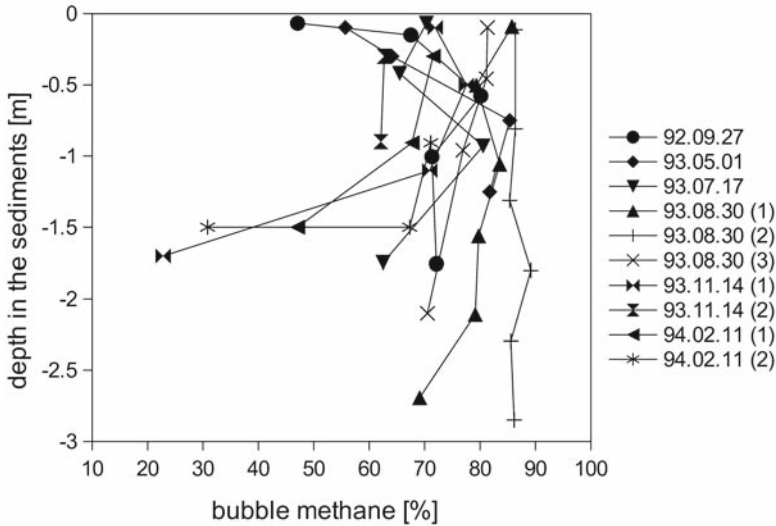
FIG. 3a. Vertical variations and seasonal cycle in the vertical variations in bubble CH_4 concentration in the Lake Moszne sediments.
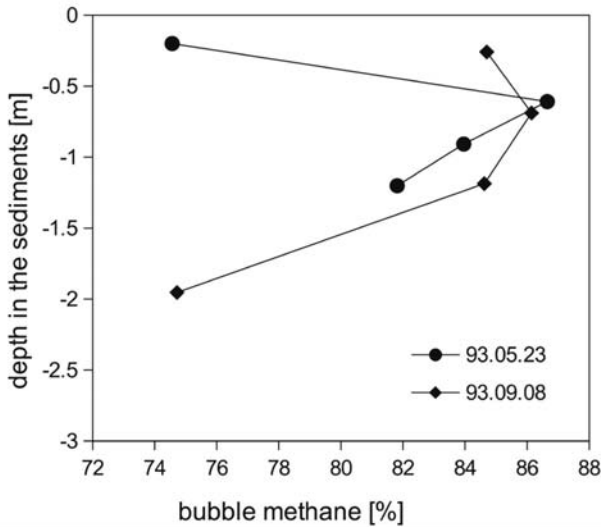
FIG. 3b. Vertical variations and seasonal cycle in the vertical variations in bubble CH_4 concentration in the Lake Skrzynka sediments.

Methane concentration in bubbles varied from 17% to 90% in the present investigations (Fig. 3ab, 6ab). CO_2 constituted from less than 1% to about 8% (Fig. 6ab), but dominantly nitrogen constituted the other components, from sev-

eral to about 80% of the gaseous bubbles. Only trace amounts of other gases were detected. In general, bubble generation by sediments weakly vary along the profiles, the methane content during summer was high in contrast to that during cold seasons (Fig. 3ab). In some profiles, especially taken in the spring and autumn (Fig. 3ab) the bubbles from mid-depth zones which are relatively rich in methane. The lowest concentration of methane has been noted in the deepest part of the cold seasons profiles (Fig. 3a).

Six profiles of the carbon isotope composition of methane in sediments from Lake Moszne are shown in Fig. 4a, and the four other profiles are shown in Fig. 4b (Lake Skrzynka). In Lake Moszne, sampling stations were about 1 m from the margin of the lake. A general trend of decreasing $\delta^{13}C$ values with increasing depth was observed in the profiles taken in 92.09.27, 93.05.01, 93.08.30 (Lake Moszne, Fig. 4a). This pattern seems to correspond to sediments temperature, especially when look on the profiles in:

- the summer (Fig. 4ab);
- upper part of the profile of the late spring (93.05.23, Fig. 4b) - the uppermost parts of sediments are already warm (Fig. 2a), and;
- lower part of the profile of the early autumn (92.09.27, 93.14.11 Fig. 4a)
- the deeper sediments are still warm (93.14.1 1, Fig. 2a).

In Lake Moszne during sampling in 93.1 1.14 and 94.02.11 the lake was frozen (10 and 15 cm of ice cover, respectively). These profiles showed trends of increasing $\delta^{13}C$ values with increasing depth (Fig. 4a).
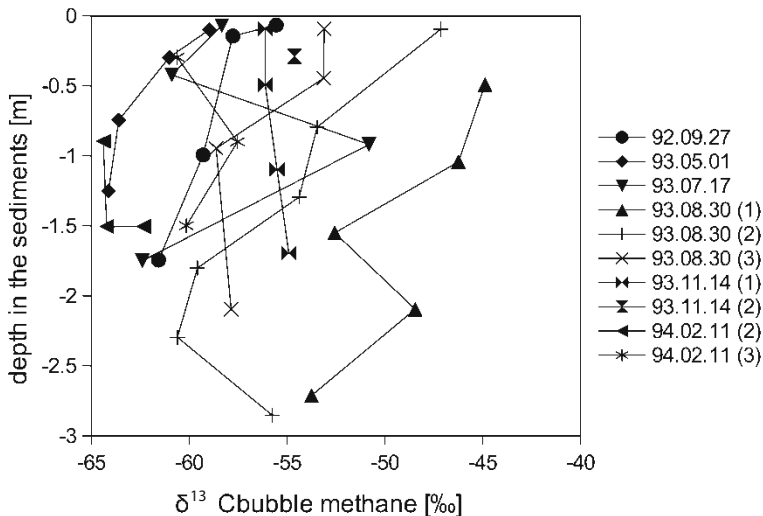

FIG. 4a. Vertical variations and seasonal cycle in the vertical variations in $\delta^{13}C(CH_4)$ value in the Lake Moszne sediments.

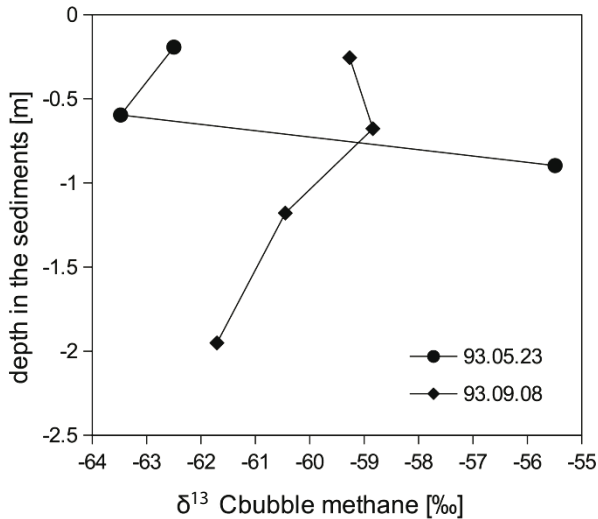**FIG. 4b.** Vertical variations and seasonal cycle in the vertical variations in $\delta^{13}C(CH_4)$ value in the Lake Skrzynka sediments.

During winter methane generation from the upper ~1m was so low that only several milliliters of gas have been released due to agitation of the sediments (whereas in the same place during the summer the same volume of sediment released approximately thousand larger volume of the gas). Thus, the uppermost sample of the 94.02.11 profile represents methane from as wide an interval of sediments as 0.0 m to 1.2 m. The autumn 1993 and 1994 profiles exhibited a rather high $\delta^{13}C$ value as compared to that in the late spring and early summer profiles, but the highest values were shown by the late summer samples and the lowest value by the late winter sample.

4. DISCUSSION

AGEING OF SEDIMENTS AND ISOTOPE FRACTIONATION
– A GENERAL OVERVIEW

In general, relatively simple, low-molecular weight compounds, such as nonstructural carbohydrates and protein-bearing materials, are more easily degraded than the complex polymeric compounds such as the lignin of woody and emergent aquatic plants (Moore 1969, Wetzel 1975, Koyama et al. 1979, Heal

and Ineson 1984). For example, Bell (1969) incubated flooded soil anaerobically and found that glucose and peptone were degraded to methane and carbon dioxide much faster than cellulose, although the total volumes of methane and carbon dioxide eventually produced from the three substrates were roughly the same. Similarly, algal material is decomposed to methane and carbon dioxide about 10 times faster than is lignocelluloses (Benner et al. 1984). Therefore, methane production should decrease with sediment maturation. In fact, tube incubations showed that the rate of methane production in freshwater lake sediments decreased from maximum values at surface to nearly zero at depths of 40 to 60 cm. Likewise, total organic carbon decreased from surficial values (5% to 2%) at 40 to 60 cm with no further decreases with depth (Koyama et al. 1979, Koyama 1990).

Blair and Carter (1992) estimated the carbon isotope fractionation factor during methane production in anoxic marine sediments to be 1.032. They have shown that the $\delta^{13}C$ value of methane produced from acetate is more negative than the $\delta^{13}C$ value of the methyl group of the acetate. Krzycki et al. (1987) have found that the carbon isotope difference between the methyl carbon of acetate and methane is 2l‰. The $\delta^{13}C$ value of CH_4 produced from acetate under steady state conditions was estimated to be similar to that of methyl carbon of acetate (-43 to -30‰) in sulfate depleted freshwater areas and the intramolecular isotope distribution of acetate was -43‰ to -30‰; for methyl carbon and -24‰ to -15‰ for carboxyl carbon (Sugimoto and Wada 1993). It was reported also that, in the CO_2-CH_4 system, the fractionation is 1.049 at 36°C (Krzycki et al. 1987) and ranges widely dependent on bacterial species; for example, at 45°C the fractionation factor varies at least from 1.045 to 1.061 (Games et al. 1978). Methane produced from other substrates than carbon dioxide and acetate, could be classified as a third group (Oremland et al. 1982) but we do not have evidence of that and its $\delta^{13}C(CH_4)$ could be different from the value for methane produced from carbon dioxide and acetate. To this group of pathways may belong for example, the production and consumption of the methyl group of methionine and dimethysulphide, methylated amines, methanol, ethanol, benzoate, reduction of carbon monoxide, formate, etc. (Zindler and Brock l978ab, Weimer and Zeikus 1978, Patterson and Hespell 1979, Oremland et al. 1982). However, only under certain special conditions this third group of methanogenic pathways does play an important role. King et al. (1983) reported that 35.1 – 61.1% of methanogenesis occurring in slurries of sulfate-rich intertidal sediments was from trimethylamine, whereas Lovley and Klug (1983) determined that 15% and 5% of total methanogenesis in low sulfate lake sediments could be accounted for methyl amines and methanol, respectively.

The factor limiting acetate fermentation is the production rate of acetate, whereas the CO_2/H_2 production of CH_4 may be controlled by H_2 transfer (Conrad and Babbel 1989). However, to be more specific, the factor limiting acetate

dissimilation is the availability of acetate. Acetate production rate can be very high, but only a little fraction is converted to methane. If hydrogen is produced in substantial amounts by bacteria and dissociation of water, and likewise the CO_2 concentration in water is relatively high, these may enhance methanogenesis via the CO_2/H_2 reduction in the uppermost horizons of sediments. The abundances of oxygen and other potentially important electron acceptors supporting the decomposition of organic matter also depend on the rates of microbial activities that are, in turn, controlled by light and temperature resulting from day-night variations (Jędrysek 1994, Jędrysek et al. 1997). Moreover, the different mechanism of biogenic methane production can result in a wide range of isotopic composition especially within millimeters to centimeters of the sediment-water interface. In general, the concentrations of CO_2 and acetate in marine sediments increase and decrease downward, respectively (Crill and Martens 1986). Both gross acetate production rate and acetate concentration in sediments is the highest in the surface layer (0–2 cm) and it is several times lower at depth of 8-10 cm, although the variation of acetate oxidation rate with depth, need to be better clarified (Christensen 1984, Michelson et al. 1989).

[14]C incubation experiments demonstrated that acetate concentration in sediments decreased substantially after long incubation periods (Michelson et al. 1989). Acetate turnover is high near the surface, but it is unsure if acetate dissimilation significantly exceed the rate of CO_2/H_2 reduction, because H_2 production is the highest near the surface as well. Despite that this point is not well documented in the literature, because of the uncertainties and potential artifacts associated with the ^{14}C-tracer acetate turnover measurements, some evidences, favoring the point that acetate dissimilation is relatively more important in the surficial sediments, comes from ^{13}C experiments. Namely, the similar situation was clearly observed in paddy soil-water incubation studies, and the $\delta^{13}C(CH_4)$ value was a useful indicator for assessing the contribution of acetate fermentation to the total production of methane: the high $\delta^{13}CH_4$ values corresponded to a higher contribution of the acetate process (Sugimoto and Wada 1993).

PROFILES ANALYZED

We speculate that the input of fresh organic matter into sediments, in the end of summer, could slightly enhance the production of acetic acid and provide the higher $\delta^{13}C$ values for methane. Consequently, the late summer/early autumn methane could show the more positive $\delta^{13}C$ value in the annual cycle. In fact, highest $\delta^{13}C(CH_4)$ values (Fig. 4a) and visually judged highest methane production were observed during late summer and early autumn. Abundant bubble methane is easily and efficiently obtained by limited stirring of sediment during summer, whereas low amounts of bubble methane are obtained in similar conditions by extensive stirring of sediments during winter. This observation corre-

lates well with annual variations of ebullition (Martens et al. 1986, Chanton and Martens 1988, Burke et al. 1988, Jędrysek et al. 1994, Jedrysek 1997) and temperature seasonal variations (Fig. 2a). Both ebullition and temperature are the lowest during winter and the highest during summer. This result is caused probably by the fact that methane is less soluble at lower temperature (Chanton et al. 1992) and by vanishingly low production of methane during winter (Jędrysek 1997).

Inasmuch as fringing vegetation of macrophytes is likely to be critical in affecting methanogenesis (e.g. Gerard and Chanton 1993), because partial consumption of methane by oxidation can significantly shift hydrogen and carbon isotopic ratios positively in the residual methane (Zyakun et al. 1979, Barker and Fritz 1981, Coleman et al. 1981). For this reason in our sampling stations no submerged or emerged macrophytes were present within several meters. Therefore, the decrease in $\delta^{13}C(CH_4)$ with increasing depth in sediments (Fig. 4ab) was possibly caused either by active methane consumption close to the surface and/or by higher contribution of acetic acid fermentation near the surface layers and a relatively greater contribution by the CO_2/H_2 reduction in deeper parts of sediments. However, anoxic conditions were observed just several centimeters below the water-sediment interface, and the sulfate concentration in pore waters was close to zero (Jędrysek 2005b). Thus, latter process, of greater contribution by the CO_2/H_2 pathway in deeper parts of sediments, is likely emphasized here, since the positive depth-$\delta^{13}C$ correlation in sediments could not possibly be a result of varying degrees of bacterial oxidation of trapped methane.

Nonetheless, despite anaerobic conditions, oxidation should not be neglected, and therefore more facts should be considered. It concern especially relations between methane concentration and $\delta^{13}C(CH_4)$ value. Decrease in methane concentration in general does not correlate to $\delta^{13}C(CH_4)$ values (Fig. 5ab) – a negative correlation should be observed if oxidation was the dominant factor controlling $\delta^{13}C$ value. On the other hand, two profiles of 93.05.01 and 94.02.11 show significant negative correlation (Fig. 5a) which may suggest apparent oxidation effect. However, these two profiles are contradictive. Namely, in the profile of 94.02.11 apparently the most oxidized methane (high $\delta^{13}C$ value) corresponds to the deep part of the profile, but in the profile of 93.05.01 apparently the most oxidized methane (high $\delta^{13}C$ value) corresponds to the surficial part of the profile. It would imply that oxidation has no relation to depth in the sediments which, on the other hand, is crucial for oxidation potential, sulfate concentration etc. Additionally, the extremely large variations in the $\delta^{13}C$ values in the profile of 93.08.30 does not correspond to any remarkable variation in the CH_4 concentration (Fig. 5a). Moreover, a negative correlation in the CO_2-CH_4 system should be observed if oxidation was important sink of methane, but contrary, a positive correlation (Fig. 6b) or no correlation has been observed (Fig. 6a).
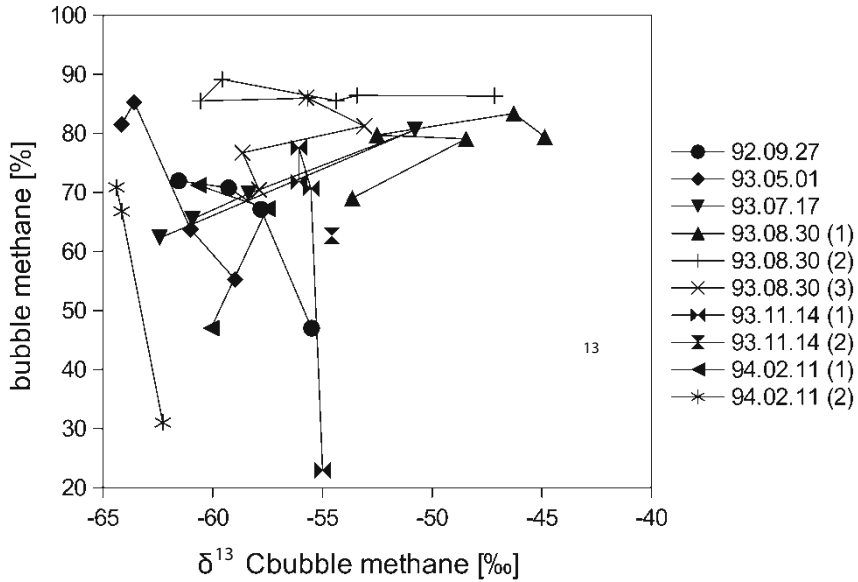
FIG. 5a. Correlation between $\delta^{13}C(CH_4)$ and concentration of bubble CH_4 concentration in the Lake Moszne sediments.
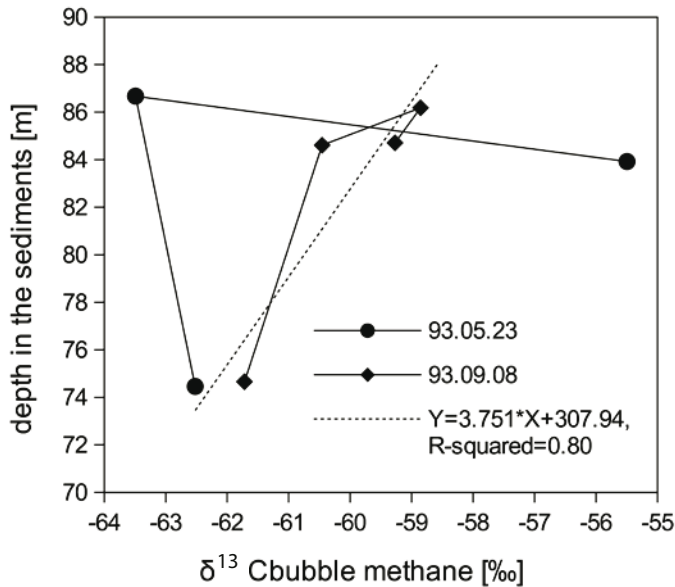

FIG. 5b. Correlation concentration between $\delta^{13}C(CH_4)$ and bubble CH_4 concentration in the Lake Skrzynka sediments.
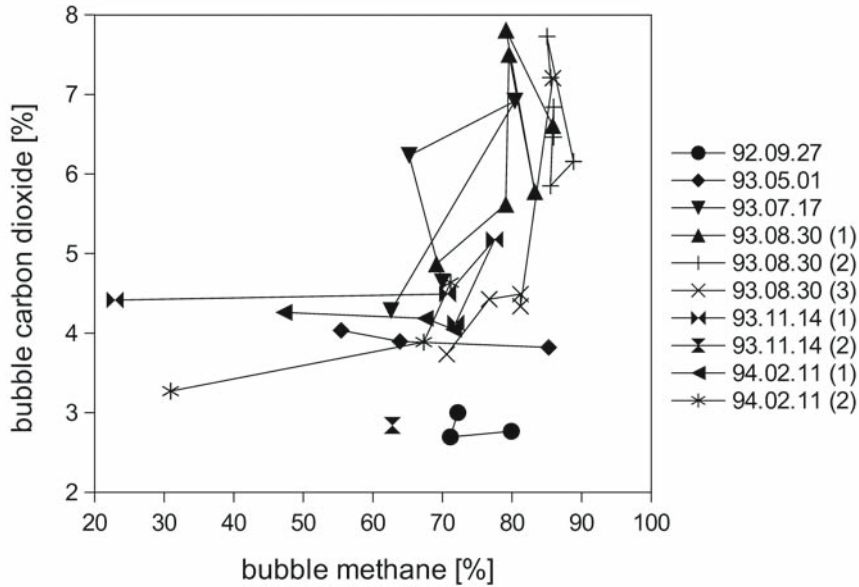
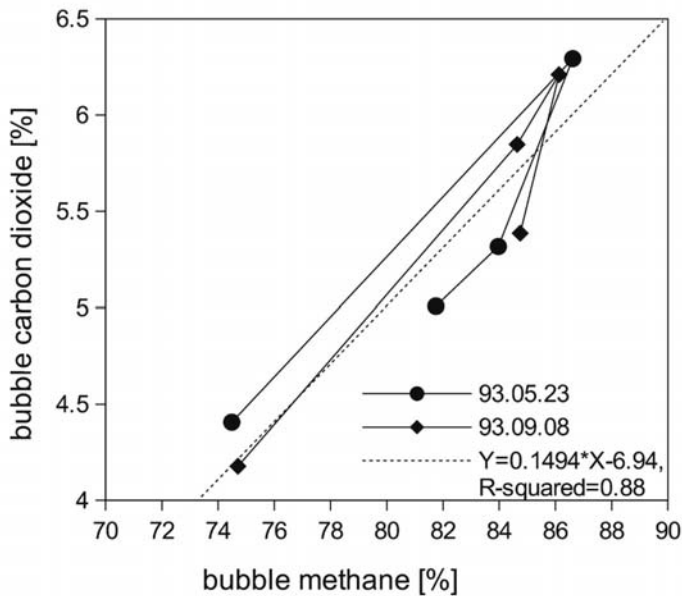**FIG. 6a.** Correlation between concentration of bubble CH_4 and concentration of bubble CO_2 in the Lake Moszne sediments.

FIG. 6b. Correlation between concentration of bubble CH_4 and concentration of bubble CO_2 in the Lake Skrzynka sediments.
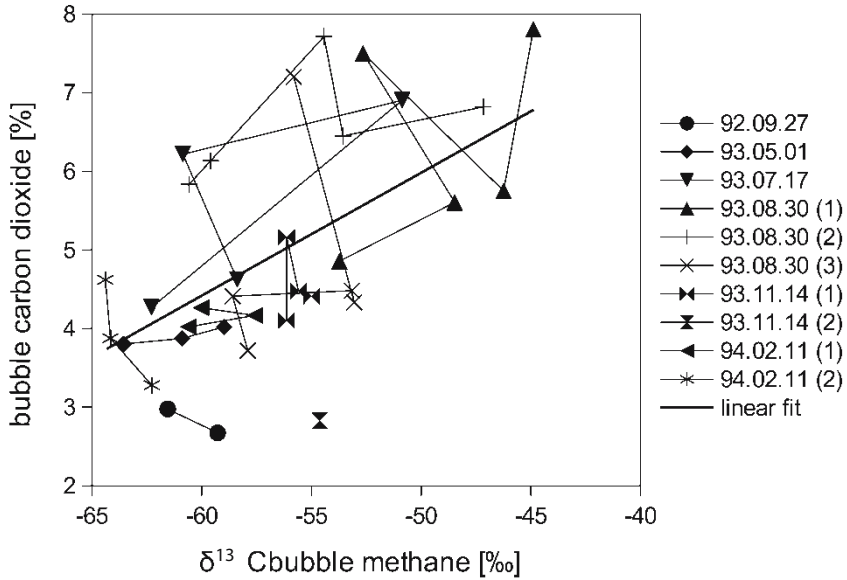
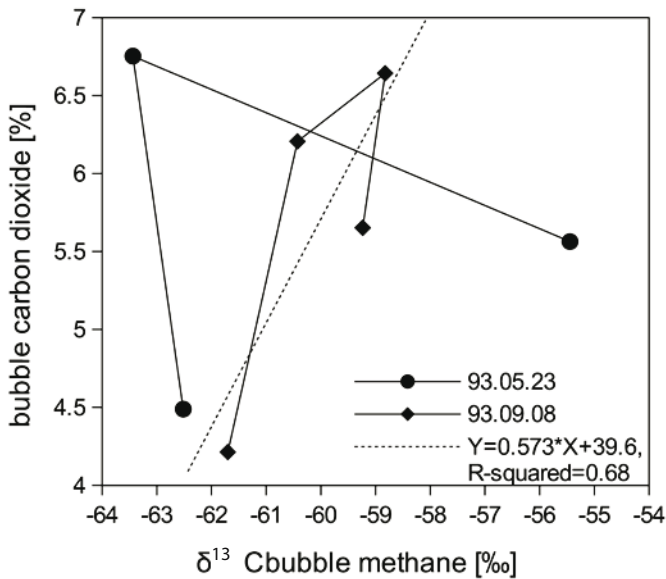**FIG. 7a.** Correlation between $\delta^{13}C(CH_4)$ and concentration of bubble CO_2 in the Lake Moszne sediments.

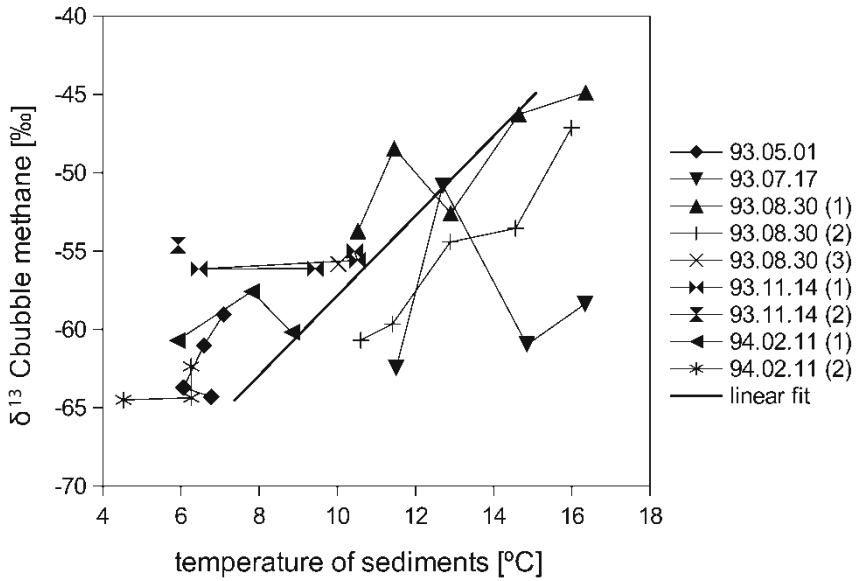**FIG. 7b.** Correlation between $\delta^{13}C(CH_4)$ and concentration of bubble CO_2 in the Lake Skrzynka sediments.

FIG. 8a. Correlation between $\delta^{13}C(CH_4)$ and temperature of sediments in Lake Moszne.

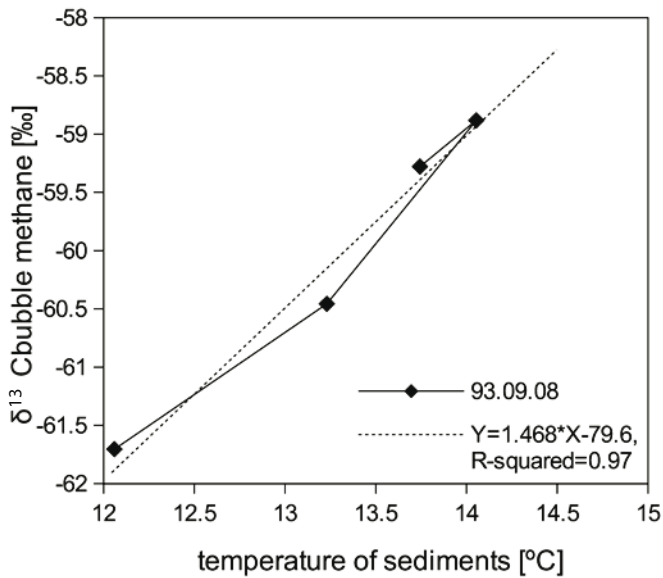**FIG. 8b.** Correlation between $\delta^{13}C(CH_4)$ and temperature of sediments in Lake Skrzynka.

However, in Lake Moszne, an increase in CO_2 concentration in bubbles poorly positively correlate with $\delta^{13}C(CH_4)$ values (Fig. 7a). Moreover, the summer profile of 93.07.17 (Fig. 7a), and especially profiles of the end of the summer i.e. 93.08.30 (Fig. 7a) and in Lake Skrzynka the profile of 93.09.08 (Fig. 7b) show a positive correlation in the CO_2-$\delta^{13}C(CH_4)$ system. This is in agreement with an expected effect of methane oxidation as important factor controlling $\delta^{13}C$ of methane. However, the same profiles are discussed below, as it shows a positive correlation in the temperature-$\delta^{13}C(CH_4)$ system (Fig. 8ab) and as it was proved above, methane in these profiles has not been oxidized. Moreover, in contrast to Lake Skrzynka (Fig. 7b), besides the mentioned above profiles, a positive correlation in the CO_2-$\delta^{13}C(CH_4)$ system, in Lake Moszne, is observed (Fig. 7a). This system seems rather complex, the DIC pool is relatively large, and many processes, not related to methanogenesis, are responsible for carbon cycling in the lake sediments. Thus, probably other factor(s), coincidentally acting in the same direction, could be responsible for this CO_2-$\delta^{13}C(CH_4)$ correlation. Probably temperature could be the crucial factor, as the winter and autumn profiles show negative or no CO_2-$\delta^{13}C(CH_4)$ correlation (Fig. 7a). In summary, we suggest that the ^{13}C-depleted methane at depth might reflect a greater contribution of methanogenesis via the carbon dioxide reduction pathway.

Changes in the relative rates of methane-producing pathways are much more probable than oxidation, and should be expected. Namely, after the acetate released in the shallower sediments was exhausted through methane production, methanogenesis could still proceed in the deeper layer, using CO_2 derived from organic and inorganic sources. Results obtained by Sugimoto and Wada (1995) convince that in contrast to acetate in freshwater sediments, at depth, the hydrogen substrates for CO_2 reduction are still present when the easily degraded organic compounds (acetate precursors) have been practically utilized. Here, an implicit assumption could be also made that acetate diffusion within the natural environment was negligible, so that all acetate produced within a given stratum was immediately consumed by reaction such as CH_4 fermentation, sulfate reduction, sorption or other processes (Sorensen et al. 1981, Christensen 1984, Michelson et al. 1989, Jędrysek 2005a).

Sebacher et al. (1986) found that methane flux from Alaskan wetlands did not correlate well with peat thickness. It would be worth to mention that this finding was consistent with our observations, based on visual judgment made in this study, and probably results from the fact that the older (deeper) sediments are not as productive in terms of methane as the younger one. In the Moszne Lake sediments, it has been observed that mainly the top 2–3 meters of organic rich sediments produced CH_4, and below 1.5 m the amount of gas bubbles released from sediments dramatically decreased. Obviously, bubble inventories will naturally decrease with depth as the sediment compacts and bubbles are forced upward. On the other hand, higher pressure at depth increases molar in-

ventory of the sediment interstices. However, any corrections for this factor are far beyond the scope of this work, as the main point here are variations of the $^{13}C/^{12}C$ ratio in methane. Anyway, in 94.02.11 visually judged highest production of methane was at the depth about 1 m, and it was very limited in the surficial zones where the temperature was about 2°C (Fig. 2a). Most probably the temperature in the upper 1 m interval was too low to develop an active methanogenesis.

Profiles of 94.02.11 and deeper parts of 93.11.14 and 93.08.30 profiles, show negative $\delta^{13}C(CH_4)$-depth correlation (Fig. 4a). The seasonal variation in vertical profiles of the $\delta^{13}C(CH_4)$ values in Lake Moszne (Fig. 4a) do not always correlate well with the corresponding seasonal variation of temperature in sediments (Fig. 2a), however, a general trend of positive correlation between temperature of sediments and $\delta^{13}C$ values have been observed in the two lakes studied (Fig. 8ab). In general, the correlation in the temperature-$\delta^{13}C(CH_4)$ system is observed only when we consider all results from several sampling actions, but not in a separate profile (Fig. 8a). The exception is the summer profile of 93.07.17 (Fig. 8a), and especially profiles of the end of the summer i.e. 93.08.30 (Fig. 8a) and 93.09.08 (Fig. 8b). In Lake Moszne the downward significant increase in temperature corresponds to a negligible downward decrease in $\delta^{13}C(CH_4)$ values in the uppermost part of the 93.11.14 $\delta^{13}C(CH_4)$ profile (Fig. 2a, 4a). The temperature and $\delta^{13}C(CH_4)$ value varies in the same direction. In the deeper part of the same profile, the downward vanishingly small decrease in temperature corresponds to the downward remarkable increase in $\delta^{13}C(CH_4)$ values. In this case, the temperature and $\delta^{13}C(CH_4)$ value varies in the opposite directions. On the other hand, the downward increase in the temperature of sediments in 94.02.11 corresponds to downward increase in $\delta^{13}C(CH_4)$ values. The temperature and $\delta^{13}C(CH_4)$ value again changes in the same direction. The 93.05.01 temperature profile shows, in the interval 0 to about 0.8 m, a downward decrease, and below ca. 0.8 m, downward increase of temperature, but the $\delta^{13}C(CH_4)$ value in this profile shows only a downward decrease, both above the 0.8 m and below 0.8 m. Therefore, temperature and $\delta^{13}C(CH_4)$ values vary independently. Hence probably no direct relation exists between temperature and $\delta^{13}C(CH_4)$ values.

However, one more case could be considered. Shifts between two separate temperature profiles and the two corresponding $\delta^{13}C$ profiles are inconsistent. For example, the temperature profile of 93.07.17 is very close to the 93.08.30 profiles (Fig. 2a – on the right side of the plot) but the corresponding $\delta^{13}C$ profile of 93.07.17 is very close to the $\delta^{13}C$ profiles of 93.05.01, 92.09.27 and even 94.02.11 (Fig. 4a – on the left side of the plot). It contradicts to the importance of temperature as a factor controlling $\delta^{13}C(CH_4)$ value in vertical profiles in sediments. Thus, another mechanism than temperature variation, probably directly influences the observed isotopic pattern.

At higher temperature of summer the rate of decomposition of organic matter in temperate climate is apparently higher than during colder seasons. The sediments studied are composed mostly of organic detritus. Temperature of the Lake Moszne sediments varied from 2°C during winter to 18°C during summer. Thus, in the surficial layers of the sediments in the lakes studied, CO_2 and acetic acid, and in the deeper regions mostly CO_2, apparently are produced most efficiently at the end of summer when in the whole profile temperature is the highest. Therefore, during this season the porewater is saturated with respect to methane precursors. It was observed by Chanton and Martens (1988) that, due to temperature-controlled solubility and temperature-dependent diffusion of methane, inventories of sedimentary gas bubbles were several times higher during summer than in winter. Thus, the observed in Lake Moszne more intensive bubble production and higher $\delta^{13}C(CH_4)$ values in the summer as compared to those in winter, were the result not only of more intensive bacterial activity at higher temperature but also of limited diffusion at higher temperature. On the other hand, it can be expected that temperature decreases results in lower concentration of methane precursors with most probably significant isotope effect. Acetate turns over so rapidly (days) that at the end of the winter, there should be vanishingly low residual pool left over from warmer seasons. Likewise, despite that the CO_2 (bicarbonate) pool is relatively large, it may be limited in the deepest part of the sediment, where the sediment is more compact and bacterial oxidation is apparently suppressed due to lower temperature. Hence, the highest ^{13}C-enrichments were observed in the deepest parts of the winter and late autumn profiles. This model may also explain the lower decreasing gradients of the $\delta^{13}C(CH_4)$ values at greater depths (93.05.0l, 93.07.17 and 93.08.30). Likewise, the surprising increase of the $\delta^{13}C(CH_4)$ values in the deepest part (below 3 m) of the 93.08.30 profile may be explained by the limited pool of CO_2 at this depth, active methanogenesis, and lower diffusion. Therefore, it can be proposed that, the deeper seated CO_2 gets isotopically heavy as more CH_4 is produced due to CO_2 pathway, and consequently CH_4 gets heavier too. When some ^{13}C-enriched CO_2 and CH_4 diffuse upwards it may result ^{13}C-enrichment in the carbon pool in the overlying levels. However, much more studies would be required to this point.

In the later stage of diagenesis, acetate and CO_2 may originate from different compounds representing different isotopic ratios, supposedly enriched in heavy carbon isotopes. Thus, the vertical variation in $\delta^{13}C(CH_4)$ not only reflects the acetate/carbon dioxide pathways and kinetic enrichment in ^{13}C of the residual precursors of methane, but also the isotope characteristics of the precursors of acetate and carbon dioxide. Further studies will be required on this point too. Particularly the variations in the $CO_2/HCO_3^-/CO_3^{2-}$ molar ratios due to increasing pressure downward within the sediment, temperature and pH and their potential influence on the $^{13}C/^{12}C$ isotopic ratios in the reduced CO_2 (the methane precur-

sor) should be considered. Such studies could provide also an important basis on which a paleoenvironmental reconstruction based on vertical variations of carbon isotope composition of carbon-bearing compounds of sediments (organic matter, carbonates) can be developed.

5. CONCLUSIONS

1. In freshwater sediments, visually judged methane production decreased with increasing depth in sediments and gradually ceased at a depth of about 2–3 meters. In the sediments studied, below about 3 m, no substantial amounts of methane were produced or released during summer. The depth of near zero production or release was about 2 m during winter. Therefore, methane flux may not correlate with sediment thickness, if the sediments in the present fields are much more than 3 m in thickness.

2. In the sediments studied, temperature and oxidation are, in general, not the factors directly responsible for the isotopic signature of methane.

3. It is proposed that the CO_2/H_2 pathway became more important with increasing depth in sediments, and methanogenesis via acetic acid fermentation decreased with increasing depth in sediments. At depths below 1 m the CO_2/H_2 pathway exclusively dominates. Probably, other pathways of methanogenesis, such as via methanol and methylamines (which is negligible from the isotopic point of view) also decrease with depth. Some oxidation of methane at shallower depths could occur but generally it is not the primary reason for the observed pattern.

4. During summer at the depth about 3 m and during winter in the entire profile, the production of methane precursors probably decreases to vanishingly low values. However, because of continuing methanogenesis, a kinetic enrichment in heavy isotopes of the residual carbon pool is responsible for the observed gradual downward decrease of the gradient of ^{13}C isotope depletion of methane. finally, this process results in downward increase of the isotope ratios of methane at the depth of about 3 m during summer, and below the depth of about 1 m during late autumn-winter.

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