LETTER

Origin of $^{13}$C-enriched methane in the crater lake Towada, Japan

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Concentration and stable carbon isotopic composition ($\delta^{13}$C) of CH$_4$ are determined in a water column of an 327 m deep oxic oligotrophic crater lake, Lake Towada in Japan. The results of CTD measurements show relatively high temperature and high conductivity in the lower part of the column, possibly derived from hot springs at the crater wall approximately 150 m deep. The vertical profile of CH$_4$ concentration shows two sharp maxima of 68 and 69 nmol/kg at depths of 20 and 150 m, respectively. The $\delta^{13}$C value of CH$_4$ at 20 m is −55‰PDB, suggesting microbial production in and around the lake. In contrast, the $\delta^{13}$C value of CH$_4$ at 150 m is +11‰PDB, which suggests some secondary isotopic alternation processes peculiar to hot spring-derived CH$_4$ in the oxic water column, such as rapid aerobic microbial oxidation.

INTRODUCTION

Freshwater environment is an interesting field to study the source and the behavior of CH$_4$. Most reports involve eutrophic or mesotrophic lakes (e.g., Jannasch, 1975; Harrits and Hanson, 1980; Oremland and Des Marais, 1983; Lidstrom and Somers, 1984; Oremland et al., 1987; Faber et al., 1996; Utsumi et al., 1998), which are abundant in dissolved CH$_4$. Reports on the behavior of CH$_4$ in an oligotrophic freshwater environment are few (Lilley et al., 1988), especially on the water column distribution of stable carbon isotopic composition ($\delta^{13}$C) of CH$_4$, which can be a good indicator to characterize source and behavior of CH$_4$. This parameter is usually expressed with reference to a standard (PDB) as follows:

$$\delta^{13}C = \left\{ \frac{\left(^{13}C/^{12}C\right)_{\text{sample}}}{\left(^{13}C/^{12}C\right)_{\text{standard}}} - 1 \right\} \times 1000 \text{ (‰)}.$$

The $\delta^{13}$C of methane in our terrestrial system vary from almost 0‰PDB to less than −110‰PDB. In general, −50‰PDB is considered the boundary between biogenic ($^{13}$C-depleted) and thermogenic CH$_4$ ($^{13}$C-enriched) (e.g., Schoell, 1983; Cicerone and Oremland, 1988; Stevens and Engelkemeir, 1988). In addition, $\delta^{13}$C of biogenic
CH₄ could be a useful tracer to distinguish two different methanogenic reaction pathways: acetate fermentation and CO₂ reduction (Woltemate et al., 1984; Sugimoto and Wada, 1993).

In oligotrophic lakes, and particularly isolated subalpine lakes, primary production and input of organic compounds and inorganic nutrients are low, so that significant anaerobic methanogenic activity in the water column is unlikely to occur. Thus, the CH₄ concentration in oligotrophic lakes is too low (less than 100 nmol/kg) for δ¹³C determination by traditional technique. Here we report concentration and δ¹³C values of CH₄ measured in a water column of an oxic (oxygenated) oligotrophic crater lake. By using a recently developed isotope-ratio-monitoring gas chromatography/mass spectrometry (irmGC/MS or GC/C/IRMS) system, we were able to make such measurements for trace amount of CH₄ (less than 10 nmolC) (Popp et al., 1995; Faber et al., 1996; Sansone et al., 1997; Tsunogai et al., 1998) to clarify the source and behavior of methane in an oxic oligotrophic, Quaternary crater lake, Lake Towada, where hydrothermally (probably thermogenic) derived CH₄ are expected with low background concentration of microbially derived CH₄.

To study the source of methane under oxic environment, we need to consider the possibility of secondary alternation by methane-oxidizing bacteria. Carbon isotopic composition of CH₄ would be altered by microbial oxidation in oxygenated water (Coleman et al., 1981; Barker and Fritz, 1981). Methane oxidation is well documented both in freshwater and marine environments by following the temporal variation of dissolved CH₄ (Jannasch, 1975; Utsumi et al., 1998) and added ¹⁴CH₄ concentrations (Harrits and Hanson, 1980; Lidstrom and Somers, 1984; De Angelis et al., 1993).

In this study, besides the contents and δ¹³C of CH₄, we measured their temporal variation in the incubated lake waters in order to evaluate microbial population within the oxic lake water column.

![Fig. 1](image-url)  
Fig. 1. Map showing the location of Lake Towada and the sampling point (★).
MATERIALS AND METHODS

Study site

Lake Towada is a Quaternary crater lake in the northeast Japan, situated in a double caldera structure 400 m above sea level. It has an area of about 59.8 km², including a 70 m deep flat basin and a steep central crater 327 m deep (Fig. 1), in which winter overturn is not perfect (meromictic). The summer stratification of water in the lake is considered to begin in May or June and continues until November or December. The water is oligotrophic and almost vertically constant in oxygen (more than 80% of air saturated concentration) and in dissolved inorganic nitrogen (ca. 0.1 mg/l), irrespective of depth or season (Shoji et al., 1985).

Hot spring activities in Lake Towada have been expected through many past limnological studies (Yoshimura, 1937; Morita, 1973) and were systematically studied in 1988 and 1994 (Nojiri et al., pers. comm.). These studies revealed several chemical enrichments associated with temperature anomalies in deep lake water below 50–100 m in the crater. Helium isotope study suggested that these anomalies were caused by a few percent contribution of high temperature mineralized water, probably derived from hot springs located at about 150 m depth in the central crater wall.

Sampling

Water samples were collected in September 1995 from the central crater of the lake (40°26.787' N, 140°54.233' E, Fig. 1). Prior to water sampling, a CTD system (Conductivity Temperature Depth recorder; SeaCat SBE19, Sea-Bird Electronic Inc.) was lowered to a depth of 320 m to measure electrical conductivity and temperature versus pressure. The measurement range, accuracy and resolution of the temperature sensor is −5 to +35°C, 0.01°C and 0.001°C, respectively. That of the electrical conductivity sensor is 0 to 60 mS/m, 100 μS/m and 2 μS/m, respectively. That of the semiconductor stain-gauge pressure transducer is 0 to 68500 hPa, 350 hPa and 35 hPa, respectively.

Samples were collected with a 2.0 L Niskin sampler at 10 or 20 m intervals. No effervescence of dissolved gas was observed for the samples. For CH₄ analyses and for methane oxidation incubation experiments, each sample was immediately divided into ca. 120 ml sterilized clean glass vials, which were flushed by sufficient overflow to prevent air contamination. The vial for the CH₄ analysis was then poisoned by adding 1.0 ml HgCl₂ saturated solution, capped with an isoprene rubber septa without headspace, and sealed with an aluminum crimp seal. The vial for the methane oxidation incubation experiments was treated similarly but without adding HgCl₂ solution, in order not to obstruct bacterial CH₄ oxidation activity (Utsumi et al., 1998). All the vials were transported to our laboratory, being kept in dark place at 5°C for two weeks before analyses, in order to evaluate CH₄ oxidation during the period.

Analysis

Methane concentration and carbon isotopic composition were analyzed simultaneously with the irmGC/MS system (Tsunogai et al., 1998). The dissolved methane was extracted by He-sparging and then treated by CO₂ removing, drying, twice condensing, GC-separation, oxidation, and water removing, before making ⁴⁵CO₂/⁴⁴CO₂ and ⁴⁶CO₂/⁴⁴CO₂ ratio measurement in a Finnigan MAT delta S isotope-ratio-monitoring mass spectrometer. The methane content in a sample was determined by comparing the measured ⁴⁴CO₂ output with that of a standard gas, which contained 100 ppm methane in a Nitrogen gas, which had been precisely calibrated by Takachiho Trading Co. Ltd. The precision (1σ value for 10 determinations) for CH₄ analysis was 6.5% at the concentration level of 2 nmol/kg, and for the isotopic analysis was estimated to be around ±2 and ±7 (‰) at the methane size of 6 and 0.7 nmol, respectively, which correspond to the concentration levels of 50 and 6 (nmol/kg), respectively, in this study. No isotopic measurement was made for the samples containing CH₄ less than 0.7 nmol.
RESULTS AND DISCUSSIONS

As shown in Fig. 2, significant increase was observed in both temperature and conductivity in deeper part below 50 m. Similar results were also observed in the previous studies (Yoshimura, 1937; Morita, 1973; Shoji et al., 1985; Nojiri et al., pers. comm.). The profiles supports the previous view that the lake is fed by an inflow of warm, mineralized hot spring fluid. Both profiles show maximum values at the depth of around 150 m, suggesting the lateral inflow at this depth.

The measured methane concentrations (Table 1 and shown as ■ in Fig. 3(a)) show two distinct maxima at depths of 20 m (68 nmol/kg) and 150 m (69 nmol/kg). The carbon isotopic composition at 20 m is $-55\%_{o}$PDB, which is within the range of microbial origin. On the other hand, pronounced $^{13}$C-enriched value of $+11\%_{o}$PDB is observed at 150 m.

Together with the temperature and conductivity profiles (Fig. 2), the deeper CH$_{4}$ maximum can be attributed to input of hot spring water from the crater wall. The carbon isotopic composition of $+11\%_{o}$PDB is, however, more $^{13}$C-enriched than usual CH$_{4}$ in subaerial hot spring waters (thermogenic CH$_{4}$: $-50$ to $-20\%_{o}$PDB) (Welhan, 1988). It is higher than the most $^{13}$C-enriched carbon isotopic composition of CH$_{4}$ so far reported in freshwater environments ($^{13}$C $< -10\%_{o}$PDB) (Oremland and Des Marais, 1983; Woltemate et al., 1984; Oremland et al., 1987; Stevens and Engelkemeier, 1988; Faber et al., 1996). It is difficult to believe that the value reflect actual source $^{13}$C composition of the hot spring water in the lake. Such an $^{13}$C enriched isotopic

![Fig. 2. Vertical profiles of temperature (broken line) and conductivity (solid line).](image)

![Fig. 3. Vertical distribution of concentration (a) and carbon isotopic composition (b) of CH$_{4}$ at Lake Towada (■), together with those of residual CH$_{4}$ after incubation experiment (○).](image)
Table 1. Results of the methane oxidation incubation experiment. Concentration and carbon isotopic composition of initial methane (initial) and that of residual methane after two weeks incubation (residual) are shown, together with calculated kinetic carbon isotope fractionation factor ($\alpha_c$).

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>Initial</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH$_4$ (nmol/kg)</td>
<td>$\delta^{13}$C (‰ PDB)</td>
</tr>
<tr>
<td>1</td>
<td>34</td>
<td>-45 ± 2</td>
</tr>
<tr>
<td>20</td>
<td>68</td>
<td>-55 ± 2</td>
</tr>
<tr>
<td>40</td>
<td>25</td>
<td>-45 ± 2</td>
</tr>
<tr>
<td>60</td>
<td>27</td>
<td>-31 ± 2</td>
</tr>
<tr>
<td>80</td>
<td>11</td>
<td>+15 ± 4</td>
</tr>
<tr>
<td>100</td>
<td>8.7</td>
<td>+28 ± 4</td>
</tr>
<tr>
<td>120</td>
<td>1.1</td>
<td>—</td>
</tr>
<tr>
<td>140</td>
<td>46</td>
<td>-9 ± 2</td>
</tr>
<tr>
<td>150</td>
<td>69</td>
<td>+11 ± 1</td>
</tr>
<tr>
<td>170</td>
<td>1.6</td>
<td>—</td>
</tr>
<tr>
<td>190</td>
<td>1.8</td>
<td>—</td>
</tr>
<tr>
<td>210</td>
<td>0.9</td>
<td>—</td>
</tr>
<tr>
<td>230</td>
<td>0.6</td>
<td>—</td>
</tr>
<tr>
<td>250</td>
<td>16</td>
<td>+31 ± 4</td>
</tr>
<tr>
<td>270</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
<td>290</td>
<td>3.3</td>
<td>—</td>
</tr>
<tr>
<td>310</td>
<td>6.8</td>
<td>-15 ± 7</td>
</tr>
</tbody>
</table>

composition could be due to the work of methane-oxidizing bacteria in the lake water column, since the bacteria prefer $^{12}$C and thus leave unoxidized CH$_4$ enriched in $^{13}$C (Silverman and Oyama, 1968; Barker and Fritz, 1981; Coleman et al., 1981; Whiticar and Faber, 1986).

For samples that were incubated for two weeks, the measured initial and residual methane concentration and carbon isotopic composition at each depth are given in Table 1. During the two-week incubation, the $\delta^{13}$C value of CH$_4$ at 150 m increased from +11 to +72‰ PDB while the CH$_4$ concentration decreased from 69 to 7.9 nmol/kg. Similar but smaller changes occurred also at 140 m. These changes in the incubated samples are also shown as ○ in Fig. 3. The calculated kinetic isotope fractionation factors of the changes (1.013 and 1.020) are comparable to values reported for the aerobic microbial methane oxidation (Barker and Fritz, 1981; Coleman et al., 1981; Whiticar and Faber, 1986).

Such changes in incubated samples indicate that active microbial CH$_4$ oxidation had been occurring in the spring-mixed, deep lake waters. The difference between CH$_4$ concentration profile and conductivity profile of spring-mixed water in the deep lake water (Figs. 2 and 3) may be due to the rapid microbial CH$_4$ oxidation. Enrichment of dissolved components in thermal spring water result in higher densities of spring-mixed lake water than ambient lake water (e.g., Chen and Millero, 1977), so that warmer spring-mixed water sink down to bottom. During accumulation of mineralized spring-mixed waters in the deep lake water, major chemical components (such as Na$^+$, Cl$^-$ etc.) are stable, so that enrichment of major chemical components (i.e., high conductivity) was observed all over the deep lake water (Fig. 2). On the other hand, dissolved CH$_4$ would be almost exhausted in aged vent-derived waters below 170 m and above 120 m, so that only young vent-derived water is observed as sharp CH$_4$ enrichment.
around 150 m (Fig. 3(a)). The $^{13}$C enrichment observed in samples below 60 m may be caused by contribution of unoxidized, trace amount of residual CH$_4$ highly enriched in $^{13}$C (Fig. 3(b)).

In contrast, little changes of concentration and $\delta^{13}$C were detected in the incubation experiments for the shallower (1, 20 and 40 m) samples with comparable initial concentration (Table 1 and Fig. 3). The methane at the depth of 20 m seems to be preserved from the secondary isotopic alternation after generation. The $\delta^{13}$C value of $-55\%_{\text{oo}}$PDB is in the range of microbial origin, possibly derived from bacterial decomposition of organic matter in sediments of the shallower flat basin (Fig. 1).

Harriss and Hanson (1980) reported that the rate of methane oxidation under oxic environment was proportional to methane concentration when the in situ concentration was <$5 \mu$mol/kg. If the system that we studied behave in a similar fashion to that observed in other freshwater systems, we could anticipate the proportional correlation between relative methane oxidation rates and methane concentrations. Slow oxidation rates for the shallower methane maximum in our study coincide well with the previous observations, because methane oxidation rates, which are extrapolated to the concentrations in our study site, were expected to be sufficiently slow (under detection) in our incubation experiments due to the low-level methane concentrations. As for the deeper maximum, however, we found evidence for the active methane oxidation under the similar low CH$_4$ environment with the shallower maximum. Our results suggest that apparent CH$_4$ concentration does not always control CH$_4$ oxidation in water.

The highest methane oxidation rate, so far reported within sea water column, is measured in a methane-enriched plume derived from seafloor hydrothermal vents (De Angelis et al., 1993). End member anoxic hot spring vent fluids, in both submarine and subaerial environments including nearby areas of Lake Towada, are known to contain large amount of methane up to mmol/kg levels (e.g., Yoshida, 1984; Welhan, 1988; Marty et al., 1993). Such an elevated concentration of emitted CH$_4$ in immediate oxic vicinity of hot spring vents may stimulate methane-oxidizing bacteria around vents. Other factors, such as elevated concentrations of suspended particles, could be also responsible for stimulating bacterial activities (Weaver and Dugan, 1972) around vents, because hot spring water is usually abundant in inorganic suspended particles such as sulfur and/or sulfide materials. The stimulated bacteria around vents may spread into the deep lake water together with hot spring water and result in abundant population of methane oxidizing bacteria in the deep lake water. On the contrary, elevated concentrations of CH$_4$ up to mmol/kg level at source region or abundant suspended particles are difficult to assume for the shallow CH$_4$ maximum. The active consumption of CH$_4$ in the deep maximum, under the similar apparent methane content with the shallow maximum, may be due to the unique factors for the hot spring derived CH$_4$.

Effect of bacterial oxidation would make it difficult to determine CH$_4$ source from its isotopic ratio as discussed above. For example, isotopic composition of thermogenic range was observed in a case in which microbial CH$_4$ had migrated into an oxic environment and become partially oxidized by methane-oxidizing bacteria. Under oxic environment, we need to be very careful when trying to determine the CH$_4$ source on the basis of isotopic data, even in low methane environments.

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