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Authors
Pack, Mary A
Heintz, Monica B
Reeburgh, William S
et al.

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Methane oxidation in the eastern tropical North Pacific Ocean water column

Mary A. Pack, Monica B. Heintz, William S. Reeburgh, Susan E. Trumbore, David L. Valentine, Xiaomei Xu, and Ellen R. M. Druffel

Department of Earth System Science, University of California, Irvine, California, USA

Abstract We report methane (CH₄) concentration and methane oxidation (MOx) rate measurements from the eastern tropical north Pacific (ETNP) water column. This region comprises low-CH₄ waters and a depth interval (~200–760 m) of CH₄ supersaturation that is located within a regional oxygen minimum zone (OMZ). MOx rate measurements were made in parallel using tracer-based methods with low-level ¹⁴C-CH₄ (LL ¹⁴C) and ³H-CH₄ (³H). The two tracers showed similar trends in MOx rate with water depth, but consistent with previous work, the LL ¹⁴C rates (range: 0.034–15 × 10⁻³ nmol CH₄ L⁻¹ d⁻¹) were systematically slower than the parallel ³H rates (range: 0.098–4000 × 10⁻³ nmol CH₄ L⁻¹ d⁻¹). Priming and background effects associated with the ³H-CH₄ tracer and LL ¹⁴C filtering effects are implicated as the cause of the systematic difference. The MOx rates reported here include some of the slowest rates measured in the ocean to date, are the first rates for the ETNP region, and show zones of slow CH₄ turnover within the OMZ that may permit CH₄ derived from coastal sediments to travel great lateral distances. The MOx rate constants correlate with both CH₄ and oxygen concentrations, suggesting that their combined availability regulates MOx rates in the region. Depth-integrated MOx rates provide an upper limit on the magnitude of regional CH₄ sources and demonstrate the importance of water column MOx, even at slow rates, as a sink for CH₄ that limits the ocean-atmosphere CH₄ flux in the ETNP region.

1. Introduction

Methane (CH₄) is a powerful greenhouse gas that plays important roles in atmospheric chemistry [Cicerone and Oremland, 1988]. The marine environment constitutes a vast reservoir of CH₄ (~10¹⁹ g carbon) [Dickens, 2003; Zhang et al., 2011], but due to highly efficient and microbially mediated CH₄ oxidation, very little marine CH₄ makes its way to the atmosphere. Current estimates indicate that only 4–15 Tg CH₄ yr⁻¹, or 0.7–2.7% of the annual total sources of atmospheric CH₄, come from ocean-atmosphere exchange [Houweling et al., 2000; Wuebbles and Hayhoe, 2002; Denman et al., 2007; Ciais et al., 2013]. Thus, aerobic and anaerobic CH₄ oxidation together nearly balance CH₄ production in the marine environment and act as a “cap” that limits the influence of this large CH₄ reservoir on the atmosphere [Reeburgh, 2007]. However, many aspects of the marine CH₄ budget are not well understood or quantified. For example, the estimates of total CH₄ oxidation in ocean waters and shallow sediments that are used to constrain marine sources range between 75 and 304 Tg CH₄ yr⁻¹ [Hinrichs and Boetius, 2002; Reeburgh, 2007]. Furthermore, the effects of environmental factors that can regulate the rate of CH₄ oxidation, including nutrient availability, temporal variations in CH₄ concentrations, and oxygen levels, are not well quantified. This lack of quantitative understanding limits the ability to estimate how past and future climate may have altered the role of the ocean as a source or sink for atmospheric CH₄.

Few measurements of CH₄ oxidation rates exist for oxic ocean waters (MOx), and the overall consumption of marine CH₄ by this process is less studied than anaerobic CH₄ oxidation [Reeburgh, 2007]. The available MOx rate measurements span several orders of magnitude (~0.001–10 nmol CH₄ L⁻¹ d⁻¹) and are insufficient to reveal the environmental factors that regulate MOx [Mau et al., 2013]. An especially important, yet unresolved issue is whether oxidation ceases when CH₄ levels reach a threshold too low to support a microbial community [Scranton and Brewer, 1978; Valentine et al., 2001; Heeschen et al., 2004; Mau et al., 2013]. Central (i.e., noncoastal) surface and deep ocean waters typically have low-CH₄ concentrations (<1–5 nmol L⁻¹), and MOx rates in these waters are therefore expected to be slow and difficult to measure accurately. Turnover times of...
14–50 years (and longer for water masses >150 years old) have been estimated for the deep ocean using water mass age and CH₄ concentrations [Scranton and Brewer, 1978; Rehder et al., 1999; Heesch et al., 2004; Keir et al., 2005]. These are quite long compared to turnover times of 5–15 days measured in waters where active CH₄ seepage occurs [de Angelis et al., 1993; Pack et al., 2011; Heintz et al., 2012]. The large volume of low-CH₄ waters in the central and deep ocean means that even slow MO₃ rates in these waters can provide an important sink in the overall marine CH₄ budget.

One reason for the lack of MO₃ rate measurements in oxic ocean waters is the challenge of measuring slow rates at low-CH₄ concentrations. Methanotrophs, bacteria capable of using CH₄ as their sole source of carbon and energy, oxidize CH₄ in oxic waters via the net reaction:

\[
\text{(R1) } \text{CH}_4 + 2\text{O}_2 \xrightarrow{\text{methanotrophic bacteria}} \text{CO}_2 + 2\text{H}_2\text{O} + [\text{cell biomass}]
\]

MO₃ rates are measured by adding ¹⁴C-CH₄ or ³H-CH₄ tracer to water samples, incubating the samples for a given period of time, then removing the unreacted CH₄ and quantifying the amount of tracer that accumulated in the products. Well-established tracer-based methods for the direct measurement of MO₃ rates use ¹⁴C-CH₄ or ³H-CH₄ tracer at high levels (abbreviated as HL ¹⁴C and ³H); they use 10⁵ Bq per 160 mL of water and measure the conversion of the CH₄ tracer to carbon dioxide (CO₂) and cell biomass (HL ¹⁴C tracer) or water (³H tracer) by decay counting [Ward et al., 1987; Valentine et al., 2001; Heintz et al., 2012]. However, to detect the resulting radioactivity in the products requires a minimum addition of CH₄ tracer to the water sample (background seawater by decay counting is ~50 Bq per 160 mL). The addition of tracer to the typical volume of water utilized (160 mL) raises CH₄ concentrations by ~2–10 nmol L⁻¹ for ³H, and ~400 nmol L⁻¹ for HL ¹⁴C [Mau et al., 2013; Valentine et al., 2001]. In the open and deep ocean regions where CH₄ concentrations are 5 nmol L⁻¹ or lower, these levels of tracer addition raise the amount of CH₄ significantly (>100 times for HL ¹⁴C), possibly accelerating the measured MO₃ rates through priming [Ward et al., 1987]. Further, the interaction of the ³H-CH₄ radioactive decay (which has a 12.3 years half-life compared to 5730 years for ¹⁴C-CH₄) with surrounding molecules and ³H-CH₄ isotope exchange (a process not observed with ¹⁴C-CH₄ tracers) can lead to high ³H method backgrounds. These backgrounds are not well quantified and may become significant at slow MO₃ rates. A recently developed low-level ¹⁴C-CH₄ method (LL ¹⁴C) [Pack et al., 2011] that raises CH₄ concentrations in samples by only 0.004 nmol L⁻¹, uses ~10⁵ times less radioactivity and accelerator mass spectrometry (AMS) to measure radioactivity, addresses the challenge of MO₃ rate measurements in low-CH₄ waters (background seawater by AMS is on the order of 0.001 Bq per 160 mL). A previous comparison of the ³H and LL ¹⁴C methods in coastal waters demonstrated good agreement at rates faster than 0.1 nmol CH₄ L⁻¹ d⁻¹, but at slower rates the LL ¹⁴C results were systematically lower than the ³H results [Pack et al., 2011]. Additional LL ¹⁴C and ³H comparisons along with controlled experiments are needed to continue establishing the LL ¹⁴C method.

In this study, we applied the LL ¹⁴C and ³H tracer methods to measure MO₃ rates in the low-CH₄ waters of the eastern tropical north Pacific (ETNP). The ETNP is an area of high, upwelling-derived surface productivity that generates large quantities of sinking organic matter [Pennington et al., 2006]. Respiration of the organic matter as it descends through the water column, combined with poor ventilation, forms a regional oxygen minimum zone (OMZ) centered near 400 m depth [Pennington et al., 2006]. The OMZ extends more than 1500 km off the Mexican coast and covers an approximate area of 5.2 × 10⁶ km² (Figure 1) [Sansone et al., 2001]. Methane concentrations in the OMZ are 79 nmol L⁻¹ at the Mexican coast and >10 nmol L⁻¹ hundreds of kilometers off the coast [Sansone et al., 2001, 2004]. Overall, the ETNP comprises the largest reported open ocean CH₄ pool (~0.3 Tg CH₄) and the OMZ provides a local maximum in CH₄ compared to open ocean surface waters of ~2–8 nmol L⁻¹ and deep ocean background waters of ~2–3 nmol L⁻¹ [Sansone et al., 2001].

Past studies of CH₄ dynamics in the ETNP region indicate that CH₄ found within the OMZ has two probable sources: CH₄ produced in situ and CH₄ advected from coastal sediments. Methane in the upper 200–400 m has depleted δ¹³C-CH₄ values (~45% to ~42%) that point to a lightly oxidized biogenic source [Sansone et al., 2001]. Hence, it is thought that shallow CH₄ is locally produced in the anaerobic microenvironments of sinking particles and organisms’ intestinal tracts, or from the decomposition of methylphosphonates as a metabolic by-product [Karl and Tilbrook, 1994; Sansone et al., 2001; Reeburgh, 2007;
Karl et al., 2008]. Methane below 400 m is enriched in $^{13}\text{C}$ (−26% to −30%) near the coast and becomes even more enriched with distance from the coast (10–15‰) [Holmes et al., 2000; Sansone et al., 2001]. Microbial CH$_4$ oxidation preferentially uses $^{12}\text{C}$-CH$_4$ and leaves the residual CH$_4$ enriched in $^{13}\text{C}$ [Coleman et al., 1981]. Thus, the deeper pool is believed to be CH$_4$ that was moderately oxidized within the OMZ while advecting from organic-rich coastal sediments to the open ocean (>1500 km). The low O$_2$ conditions in the OMZ are hypothesized to slow MO$_x$ and allow CH$_4$ to travel long distances [Sansone et al., 2001, 2004].

While a number of studies have focused on CH$_4$ dynamics in the ETNP region [Burke et al., 1983; Holmes et al., 2000; Sansone et al., 2001, 2004], to date no direct measurements of MO$_x$ rates have been reported and the hypotheses those studies generated about low MO$_x$ in the OMZ or how MO$_x$ affects the potential flux of CH$_4$ from ocean to atmosphere in this region remain untested. A number of similar oxygen minimum zones exist in the marine environment and these zones may expand in response to changes in ocean circulation and anthropogenic macronutrient inputs [Naqvi et al., 2010]. A sound understanding of CH$_4$ dynamics in oxygen minimum zones will be essential in predicting potential future changes in CH$_4$ pool size and CH$_4$ emissions from these zones [Naqvi et al., 2010].

2. Methods
2.1. Sampling

Samples were collected inside the ETNP (Figure 1) during a December 2008 cruise aboard the R/V Knorr. Water samples were collected in 10 L Niskin bottles attached to a Rosette with a conductivity-temperature-depth (CTD) package (Sea-bird SBE 9) from eight stations in the ETNP (Figure 1 and Table 1). Samples for CH$_4$ concentration and $^3$H-CH$_4$ oxidation rate measurements were transferred directly from the Niskin bottles to 160 mL glass serum bottles (Wheaton No. 223748) using Tygon tubing. The bottles were filled from the bottom, flushed with two volumes of water, and sealed with gray butyl stoppers (Wheaton No. W224100-193) and aluminum crimp caps (Wheaton No. 224178-01). After sealing, each sample was inspected to ensure it was free of bubbles. Two samples from each depth were taken for LL $^{14}$C-CH$_4$ oxidation rate measurements. Samples were taken directly from the Niskin

| Table 1. Stations Occupied in the ETNP Region During This Study |
|---|---|---|
| Station | Latitude (°N) | Longitude (°W) |
| 1 | 13.023 | 104.992 |
| 2 | 12.232 | 101.229 |
| 3 | 11.624 | 99.845 |
| 4a | 11.009 | 97.480 |
| 5 | 10.69 | 96.944 |
| 6 | 9.999 | 93.722 |
| 7 | 9.521 | 91.954 |
| 8 | 8.999 | 90.001 |
bottles in 120 mL glass serum bottles (Wheaton No. 223747) previously washed with methanol, 5% hydrochloric acid, and then deionized water. The bottles were rinsed three times with sample water, filled from the bottom to overflowing using Tygon tubing and then sealed as described above. MO$_4$ rate measurements were made at all stations occupied using the $^3$H-CH$_4$ tracer but only at Stations 1, 3, 6, and 8 (Figure 1) with the LL $^{14}$C-CH$_4$ tracer, because the method required more processing time per sample.

### 2.2. Analytical Methods

#### 2.2.1. Oxygen and Methane Concentrations

Measurements of O$_2$ concentration were made with an SBE 43 sensor on the CTD package and verified with Winkler titrations throughout the cruise. After collection, samples for CH$_4$ concentration analysis were killed with 0.5 mL of aqueous saturated mercuric chloride solution and shaken vigorously. Then, a 10 mL headspace of ultrahigh-purity nitrogen gas (UHP N$_2$) was introduced by displacement of an equivalent amount of water. Finally, samples were placed upside down in boxes to prevent gas leakage through sample stoppers during transport and storage. On shore, the sample headspaces were analyzed for CH$_4$ concentration in two 3 mL aliquots using a gas chromatograph equipped with a flame ionization detector (GC 14A; Shimadzu). The results were corrected for the amount of CH$_4$ still dissolved in solution with Bunsen solubility coefficients [Yamamoto et al., 1976] calculated from room temperature at the time of analysis and sample salinity. The precision based on duplicate samples was on average ±11%. Air-equilibrated CH$_4$ solubility was estimated using the Yamamoto et al. [1976] Bunsen coefficients based on in situ salinity and potential temperature and an atmospheric CH$_4$ mixing ratio of 1.8 μL L$^{-1}$. Percent saturation was calculated by comparing the measured CH$_4$ concentrations to the air-equilibrated concentrations.

#### 2.2.2. $^3$H-CH$_4$ Oxidation Rate Measurements

MO$_4$ rate measurements with the $^3$H-CH$_4$ tracer were made as described in Valentine et al. [2001] and Heintz et al. [2012]. In a shipboard radiation van, the 160 mL water samples were injected with 50 μL aliquots of $^3$H-CH$_4$ (370 kBq, 6360 kBq mg$^{-1}$ tracer, 4 μmol L$^{-1}$ CH$_4$ in N$_2$; American Radiolabeled Chemicals) and incubated in the dark for 24 h. Samples were incubated in a refrigerator (6°C), an incubator with temperature control (13–19°C) or at room temperature regulated by air conditioning (21°C). Temperature was measured with an alcohol thermometer kept with the incubating samples and recorded at the start of the incubations. After incubations were complete, the stoppers and 60 mL of water were removed from the sample bottles, and samples were sparged with UHP N$_2$ for 30 min to remove unreacted CH$_4$. Finally, samples were resealed and stored for transport back to shore.

In a shore-based laboratory, the quantity of $^3$H-H$_2$O in each sample was measured by liquid scintillation counting. Procedure blanks were determined by counting untreated seawater, and the blank values were subtracted from the rate samples. A fractional turnover rate (k) was calculated by dividing the fraction of $^3$H-CH$_4$ tracer that was converted to $^3$H-H$_2$O by the incubation time. MO$_4$ rates ($R_{oxd}$) were calculated as the product of k and the in situ CH$_4$ concentration ([CH$_4$]) assuming first-order kinetics:

$$R_{oxd} = k \times [CH_4]$$

Methane turnover times ($\tau$) with respect to oxidation were calculated as the inverse of k:

$$\tau = 1/k$$

Killed controls were taken (1–2 per CTD cast) to spot check that nonbiological processes did not incorporate the $^3$H-CH$_4$ tracer into the aqueous phase and to test for impurities in the $^3$H-CH$_4$ that may remain in the samples after purging. Killed controls were treated with 0.1 mL of aqueous saturated mercuric chloride solution prior to injection with $^3$H-CH$_4$ and then subjected to the procedures outlined above.

#### 2.2.2. $^{14}$C-CH$_4$ Oxidation Rate Measurements

Preparation and Activity of the LL $^{14}$C-CH$_4$ Tracer and $^{14}$C-Free CO$_2$. A diluted form of the $^{14}$C-CH$_4$ tracer used in Pack et al. [2011] was employed here; an aliquot of the Pack et al. tracer was diluted by a factor of 115 with $^{14}$C-free CO$_2$ (Matheson Gas, anaerobic grade) in a preevacuated 6 L stainless canister. The activity of the resulting $^{14}$C-CH$_4$ tracer was determined using the methods outlined by Pack et al. [2011] and found to be 0.00013 ± 0.00001 kBq per 50 μL of tracer (5.4 ± 0.4 × 10$^{-14}$ moles $^{14}$C per 50 μL tracer or 0.00139 ± 0.00009 kBq mg$^{-1}$ tracer). The $^{14}$C-free CO$_2$ used with background samples was prepared by adding the same CO$_2$ as used with the tracer (Matheson Gas, anaerobic grade) to a preevacuated 2 L stainless canister.
Shipboard 14C Labeling. Samples for LL 14C rate measurements were processed using a modified version of the method described in Pack et al. [2011] and in a separate radiation van from that used for the 3H measurements. The equipment used with the 3H rate samples had previously been exposed to HL 14C tracers, so in order to avoid contamination of the LL 14C samples with residual HL 14C tracer, it was important to completely separate the processing of the 3H and LL 14C samples. One of the two 120 mL water samples collected from each depth was injected with 50 μL of 14C-CH4 (0.00013 kBq, 0.00139 kBq mg−1 tracer, 0.0086 μmol L−1 CH4 in 14C-free CO2), while the other was treated with 50 μL of pure 14C-free CO2. This treatment yielded sets of labeled and background samples. Following injection, samples were shaken to facilitate CH4 dissolution and incubated in the dark for 36 h. Samples were incubated in a shipboard refrigerator or inside coolers kept at room temperature (regulated by air conditioning). Temperatures were measured and recorded through the entire incubation with temperature data loggers (Onset Computer Corporation No. TBI32-05 + 37).

After incubation, samples were filtered to collect cell biomass, killed, and purged of unreacted CH4. For filtering, we used procedures that collected the cell biomass without exposing sample filtrate to atmospheric CO2, which can alter the sample 14C-CO2 content. First, 60 mL of sample was transferred to a syringe by displacement with an equivalent amount of UHP N2. Next, a syringe filter holder (EMD Millipore Corporation No. SX0002500) with a 1.2 μm nominal pore size quartz fiber filter (25 mm diameter, SKC No. 225-1824) was attached to the syringe with sample, and the sample was vacuum filtered into a sealed 120 mL serum bottle. The serum bottle receiving the filtrate was previously capped with a gray butyl stopper, purged with UHP N2 and filled with 0.4 mL of aqueous sodium hydroxide solution (saturated and carbonate free). The sodium hydroxide (NaOH) killed any bacteria in the filtrate and preserved the 14C-CO2 gas oxidation product as carbonate ions (CO32−) in solution. The unreacted CH4 was then removed from the sample filtrate by purging with UHP N2 for 40 min. The purging was performed with sealed bottles using two needles: one 16 gauge, 10 cm needle (Air-Tite Products No. N164) inserted into the bottom of the sample bottle to deliver the N2, and a 23 gauge, 2.5 cm needle (Fisher Scientific No. 14-826-6B) inserted in the sample headspace as a vent for the stripped gases and N2. After purging was complete, the gray butyl sample stoppers were replaced with blue butyl stoppers (Bellico Glass No. 2048-11800) in a homemade glove chamber with UHP N2 flow. The blue butyl stoppers provided a superior seal for sample transport and storage. The sealed samples were stored upside down in boxes and transported back to shore.

The quartz filters with the cell biomass were partially dried by vacuum, rolled up, and inserted into 5 cm long, 6 mm diameter, prebaked quartz tubes with stainless steel tweezers and completely dried on a 60°C hotplate for 1–2 h. The quartz tubes with dry filters were capped with clean aluminum foil and column caps (Supelco No. 20439), transported back to shore, and stored at −10°C until 14C-AMS analysis.

Samples for killed controls were collected once per CTD cast to spot check tracer purity, abiotic incorporation of tracer and NaOH killing efficacy. The samples were treated with NaOH before or within 30 min of the 13C-CH4 injection, incubated, and treated as described above.

Shore-Based 14C-AMS Analyses. Shore-based measurements quantified the 14C in the dissolved inorganic carbon (DIC: CO2, carbonic acid, bicarbonate, and carbonate) and cell biomass that accumulated in samples during incubation with the LL 14C-CH4 tracer. DIC was extracted from 14C-labeled background and killed control samples and the 14C-content measured at the University of California, Irvine Keck Carbon Cycle AMS facility as described in Pack et al. [2011]. However, our 14C-labeled samples did not require dilution with 13C-free CO2 prior to analysis because they were well below the maximum AMS detection limit (~8 times modern under the standard operating conditions at the Keck Carbon Cycle AMS facility). Procedure blanks were determined from a 14C-free DIC standard (calcite in DIC-free seawater) prepared and extracted in parallel with DIC samples and then subtracted from all DIC samples.

The cell biomass on the quartz filters was prepared for 14C-AMS analysis via double tube combustions. The tubes in which the filters had been stored frozen were cleaned with methanol and placed inside 18 cm long, 9 mm diameter quartz combustion tubes containing 0.28 mg acetonilide, 80 mg cupric oxide, and silver wire (1 mm in diameter, ~3 mm long). Acetonilide was used as a dead carbon (14C-free) carrier, because the filters contained <0.03 mg of carbon, and at least 0.1 mg is necessary for a standard AMS measurement. The combustion tubes containing the filters were evacuated, flame-sealed, and
Table 2. Summary of Incubation Temperatures for the LL $^{14}$C and $^3$H Rate Samples

<table>
<thead>
<tr>
<th>Station</th>
<th>Sample Depth (m)</th>
<th>Number of Samples$^a$</th>
<th>Incubation Temperature $^{14}$C (°C)</th>
<th>$^3$H (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6–21</td>
<td>2</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>48–58</td>
<td>2</td>
<td>23</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>68–2476</td>
<td>14</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>5–113</td>
<td>7</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>132–201</td>
<td>2</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>26–59</td>
<td>3</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>73–374</td>
<td>4</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>1</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td>9</td>
<td>33–264</td>
<td>5</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>10</td>
<td>527–2470</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

$^a$The number of rate samples that are within the listed depth range.

\[
^{14}C_{DIC} = \left(^{14}C_{LDIC} - ^{14}C_{BDIC}\right) \times \text{DIC} \times V_f
\]

\[
^{14}C_{CM} = \left(^{14}C_{LCM} - ^{14}C_{BCM\ avg}\right) \times \text{CO}_2 \times V_f / V_f
\]

Here $^{14}C_{LDIC}$ and $^{14}C_{BDIC}$ are the $^{14}$C/$^{12}$C ratios of the DIC in the labeled and background samples, respectively; DIC is the sample DIC concentration ($\Sigma$CO$_2$); and $V_f$ is the sample volume. $V_f$ is the volume of sample filtered; CO$_2$ is the moles of CO$_2$ recovered from filter combustion with acetonilide; and $^{14}C_{LCM}$ and $^{14}C_{BCM\ avg}$ are the $^{14}$C/$^{12}$C ratios of the cellular material-acetanilide mixture in labeled samples and averaged background samples, respectively. Next, the fractional turnover rate ($k$) was calculated using the moles of LL $^{14}$C-CH$_4$ added to each sample ($^{14}$CH$_4$):

\[
k = \frac{\left(^{14}C_{ADIC} + ^{14}C_{CM}\right) / ^{14}C_{CH_4}\text{ incubation time}}{14}C_{CH_4}
\]

MO$_x$ rates ($Q_{\text{oxid}}$) and turnover times ($\tau$) were calculated according to equations (1) and (2) above. Lastly, the fraction of the $^{14}$C-CH$_4$ consumed that was allocated to cell biomass ($P_{CL}$) was calculated:

\[
P_{CL} = 100 \times \frac{^{14}C_{CM}}{\left(^{14}C_{ADIC} + ^{14}C_{CM}\right)}
\]

2.2.4. $Q_{10}$ Correction for MO$_x$ Rates

Incubation temperature is an important factor in MO$_x$ rate measurements [Heintz, 2011], and every effort was made to incubate parallel-processed LL $^{14}$C and $^3$H samples at the same temperature and near in situ temperatures. Nonetheless, a malfunctioning internal thermometer and limited refrigerator/incubator facilities in the radiation vans aboard ship resulted in some significant differences. As summarized in Table 2, incubation temperature differences for parallel LL $^{14}$C and $^3$H samples ranged from 0 to 10°C. Differences between in situ and incubation temperatures were 0 to 12°C (median: 5°C) for both the LL $^{14}$C and $^3$H samples. In an effort to account for these differences and present rates that are more representative of in situ conditions, we used a $Q_{10}$ calculation to estimate what each LL $^{14}$C and $^3$H rate would be, had samples been incubated exactly at in situ temperatures (see equation (51) and Text 51 in the supporting information for the calculation details). The corrections to our rates ranged from a factor of 1 (no correction) to 2.4, with a median of 1.5, and only a few samples were corrected by a factor $>2$. We present only the $Q_{10}$-corrected rates in sections 3 and 4 below.

3. Results

3.1. Dissolved Oxygen, Methane Concentrations and MO$_x$ Rates

Dissolved oxygen profiles (Figures 2 and 3) show an OMZ, centered around 400 m depth that is typical for the ETNP region [Burke et al., 1983; Sansone et al., 2001]. Oxygen levels were saturated in surface waters and decreased below the mixed layer to 2 $\mu$mol L$^{-1}$ by 300 m depth. The OMZ extended to ~750–800 m depth, with O$_2$ levels increasing in deeper waters (Figure 3).
Methane concentration profiles (Figures 2 and 3) had two consistent features. The first was a subsurface maximum typical of open ocean waters [Lamontagne et al., 1973; Burke et al., 1983; Reeburgh, 2007] with CH$_4$ concentrations of 4.6–7.9 nmol L$^{-1}$ (189–367% supersaturated with respect to the atmosphere). The second feature was a midwater maximum centered in the OMZ with CH$_4$ concentrations of 8.6 to 19 nmol L$^{-1}$ (317–709% supersaturated) and observed in previous studies [Burke et al., 1983; Sansone et al., 2001, 2004]. At Station 1, the midwater maximum extended into the subsurface maximum, whereas at Station 8 the two maxima were well separated. This suggests that the midwater maximum may influence CH$_4$ in the mixed layer and surface waters in some areas of the ETNP. Below the OMZ (>750–800 m), CH$_4$ concentrations transitioned to undersaturated levels and reached a minimum value of 0.27 nmol L$^{-1}$ (8% saturated).

The MO$_x$ rates in this study ranged from 0.000034 to 0.015 nmol CH$_4$ L$^{-1}$ d$^{-1}$ for the LL $^{14}$C method and 0.000098–4.0 nmol CH$_4$ L$^{-1}$ d$^{-1}$ for the $^3$H method. Profiles of MO$_x$ rates (Figures 4 and 5) showed highest values near the subsurface CH$_4$ maximum: the LL $^{14}$C rates increased to 0.0016–0.015 nmol CH$_4$L$^{-1}$ d$^{-1}$, while the $^3$H rates reached 0.17–40 nmol CH$_4$L$^{-1}$ d$^{-1}$. Higher MO$_x$ rates were also present in the OMZ near the midwater CH$_4$ maximum, with rates reaching 0.010–0.012 nmol CH$_4$L$^{-1}$ d$^{-1}$ and 0.048–0.17 nmol CH$_4$L$^{-1}$ d$^{-1}$ for the LL $^{14}$C and $^3$H methods, respectively. These higher MO$_x$ rates were bounded, above and below, with lower MO$_x$ rates that reached minimum values of 0.000022 nmol CH$_4$L$^{-1}$ d$^{-1}$ (LL $^{14}$C) and 0.0019 nmol CH$_4$L$^{-1}$ d$^{-1}$ ($^3$H). Below the OMZ, MO$_x$ rates slowed to <0.00046 nmol CH$_4$L$^{-1}$ d$^{-1}$ (LL $^{14}$C) and <0.12 nmol CH$_4$L$^{-1}$ d$^{-1}$ ($^3$H).
The fraction of $^{14}$C-$\text{CH}_4$ allocated to cell biomass ($P_{\text{CL}}$, equation (6)) ranged from 0.52% to 7.8% in 10 samples with turnover times < 700 days ($\tau$, equation (2)) but was not detectable in other samples with longer turnover times. The quartz filters containing the cell biomass must have $> 4 \times 10^{-19}$ moles $^{14}$C for the AMS analysis used here, and filters from samples with turnover times > 700 days did not contain enough $^{14}$C.

### 3.2. Comparison of the Low-Level $^{14}$C-$\text{CH}_4$ and $^3$H-$\text{CH}_4$ Rates

As is readily apparent, the two tracer methods, although yielding similar patterns of $\text{MO}_x$ rates with depth, differed in absolute values. The $\text{MO}_x$ rates obtained with LL$^{14}$C were systematically lower than those measured using $^3$H (Figures 4 and 5). Forty-four of the LL $^{14}$C rates were 2–184 (median: 16) times slower than the parallel $^3$H rates, while one LL $^{14}$C rate was 1.6 times faster (Figure 6).

### 3.3. Duplicate and Killed Control Rate Samples

The mean coefficient of variation from $^3$H rate measurements on 112 pairs of duplicate samples was 18% (range: 0.6–91%). For the LL $^{14}$C rate measurements, 10 pairs of duplicate samples gave a mean coefficient of variation of 10% (range: 0.2–36%). The error associated with each method was also estimated by combining analytical errors from sample processing (e.g., decay counting, $^{14}$C-AMS, procedure blanks, sample volume, etc.) in error propagation equations. These analytic errors, withstanding the $Q_{10}$ correction, were on average 17% for both the $^3$H and LL $^{14}$C methods for the range of rates measured with each method in this study. Incorporating the $Q_{10}$ error in the propagation is not straightforward because of method artifacts and unanticipated variability in incubation temperatures attributed to incubator cooling cycles (see supporting information Text S1). Nevertheless, a conservative estimate of propagated error including the $Q_{10}$ correction gives an average error of 56% ($^3$H) and 59% (LL $^{14}$C).

Killed control samples from the $^3$H rate measurements yielded rates that were 0.2–9.4% of their corresponding rate samples and are consistent with past studies [Valentine et al., 2001; Heintz et al., 2012]. LL $^{14}$C killed control samples yielded rates that were 13–64% of their corresponding rate samples, and killed controls that were poisoned with NaOH 2–33 min after injection with $^{14}$C-$\text{CH}_4$ tracer showed higher rates than those killed before injection. These high killed controls show that microbial activity in samples was not completely stopped when the $^{14}$C-$\text{CH}_4$ was added and that NaOH in killed controls must be introduced well before the $^{14}$C-$\text{CH}_4$ tracer. Abiotic uptake of $^{14}$C-$\text{CH}_4$ tracer or impurities in the tracer could also cause high killed controls, but previous work with the same batch of $^{14}$C-$\text{CH}_4$ tracer [Pack et al., 2011] excludes this possibility.
4. Discussion

4.1. Performance of the LL $^{14}$C and $^3$H-CH$_4$ Methods

The MO$_x$ rates we report here (0.000034–4.0 nmol CH$_4$ L$^{-1}$ d$^{-1}$) include some of the lowest rates measured in the marine environment (the bulk of previously reported rates are $\sim$0.001–10 nmol CH$_4$ L$^{-1}$ d$^{-1}$) [Mau et al., 2013]. Also, the systematic difference between the LL $^{14}$C and $^3$H parallel rate measurements reported here (i.e., LL $^{14}$C < $^3$H rates) is consistent with the previous study where these methods were compared in coastal waters [Pack et al., 2011]. In that study, the two tracer methods were in agreement when MO$_x$ rates were above 0.1 nmol CH$_4$ L$^{-1}$ d$^{-1}$ but diverged when below, with LL $^{14}$C rates consistently slower than $^3$H rates. In this study, the LL $^{14}$C rates ranged from 0.000034 to 0.015 nmol CH$_4$ L$^{-1}$ d$^{-1}$ and were well below the 0.1 nmol CH$_4$ L$^{-1}$ d$^{-1}$ mark reported in the previous study.

The fraction of LL $^{14}$C tracer converted to cell biomass ($P_{CL}$, equation (6)) reported here (0.52%–7.8%) was low compared to some previous studies in the marine environment. Using the HL $^{14}$C-CH$_4$ tracer, previous studies have reported $P_{CL}$ values of 2%–66% [Griffiths et al., 1982; Ward et al., 1987; de Angelis et al., 1993], with one study reporting a maximum $P_{CL}$ of 80% [Ward et al., 1989]. These studies used filters with smaller pore sizes of 0.2–0.45 µm (compared to our 1.2 µm), so some of our low $P_{CL}$ values may have been due to cells that passed through our filters. However, another HL $^{14}$C study in low-CH$_4$ open ocean waters was unable to detect $^{14}$C in the cell fraction of any of their samples via 0.45 µm pore size filters [Jones, 1991], indicating that $P_{CL}$ values can vary greatly between environments.

Priming and method backgrounds associated with the $^3$H-CH$_4$ tracer likely led to falsely high $^3$H rates at ETNP conditions (see section 1) and contributed to the systematic difference between our $^3$H and LL $^{14}$C parallel rates. Because the LL $^{14}$C method was specifically designed for low-CH$_4$ waters with slow MO$_x$ rates, it

Figure 4. Profiles of methane, and LL $^{14}$C and $^3$H MO$_x$ rates in the upper 400 m of the water column for Stations 1–8. The error bars for the LL $^{14}$C rates are combined analytic errors. The error bars on the $^3$H rates are a fixed 18% error based on duplicate samples taken during this study. The error bars for both measurements mostly lie within the data symbols and do not include error from the $Q_{10}$ correction.
adds ~300 times less CH
\textsubscript{4} to samples and uses ~10\textsuperscript{6} times less radioactivity than the \textsuperscript{3}H method. This obviates concerns about priming and background effects. Incubation temperature is also an important factor in parallel rate mismatch [Pack et al., 2011], but we made a Q\textsubscript{10} correction to the rate data to account for this issue (see section 2). In this study specifically, the large filter pore size (1.2 \textmu m) may have led to falsely low LL\textsuperscript{14}C rates and also contributed to the systematic difference between our parallel rates. However, this effect at its maximum (assuming zero incorporation of tracer into cells, when it should have been 66\%) would have reduced the LL\textsuperscript{14}C rate values by a factor of 3. This cannot account for the maximum 184-fold difference (or even the median 16-fold difference) obtained between the two methods.

The combination of the parallel LL\textsuperscript{14}C and \textsuperscript{3}H rates from this study and the previous coastal study show a well-defined trend, albeit with some scatter, of a greater difference between methods at slower MO\textsubscript{x} rates (Figure 6). Disagreement between parallel rate measurements has also been reported for the HL\textsuperscript{14}C-CH\textsubscript{4} and \textsuperscript{3}H-CH\textsubscript{4} tracers. A study in the Black Sea reported HL\textsuperscript{14}C rates that were 0.1–350 (median: 2.1) times faster than the parallel \textsuperscript{3}H rates (Figure 6, data from Reeburgh et al. [1991]). A more recent study in an Arctic fjord observed \textsuperscript{3}H rates > HL\textsuperscript{14}C rates in ~80 nmol L\textsuperscript{-1} CH\textsubscript{4} waters, but HL\textsuperscript{14}C rates > \textsuperscript{3}H rates in waters with 20 nmol L\textsuperscript{-1} CH\textsubscript{4} [Mau et al., 2013]. The study suggested that the 440–540 nmol L\textsuperscript{-1} increase in CH\textsubscript{4} from the HL\textsuperscript{14}C tracer (as opposed to the 1–2 nmol L\textsuperscript{-1} increase from the \textsuperscript{3}H tracer) led to priming in the 20 nmol L\textsuperscript{-1} CH\textsubscript{4} waters. While in the 80 nmol L\textsuperscript{-1} waters, the enzyme machinery associated with MO\textsubscript{x} was apparently at a lower concentration than in the 20 nmol L\textsuperscript{-1} waters and became saturated with CH\textsubscript{4} from the HL\textsuperscript{14}C tracer addition. This situation yielded \textsuperscript{3}H rates > HL\textsuperscript{14}C rates (see the discussion in Mau et al. [2013]).

When planning future water column MO\textsubscript{x} rate measurements, the \textsuperscript{3}H, LL\textsuperscript{14}C, and HL\textsuperscript{14}C methods are all available tools, and their respective strengths and weaknesses should be considered.
The advantage of the $^3$H method is that samples can quickly be prepared and rapidly analyzed with liquid scintillation counting; however, it requires specialized radioisotope facilities (radiation vans) in the field. By contrast, the LL$^{14}$C method avoids the use of radiation vans but is costlier and requires more laboratory processing time than the $^3$H method. Taking these strengths and weaknesses together, the $^3$H method is best for surveys where elevated rates are anticipated, radiation vans are available, and a large number of samples need to be processed quickly, while the LL$^{14}$C method is more appropriate for surveys where priming is a concern and high-level radioactive tracers are not practical due to a lack of radioisotope facilities (remote field sites and rapid response situations).

Figure 7. Methane concentrations versus density showing that the midwater CH$_4$ maximum at different stations falls on a similar density surface highlighted in gray.

An additional advantage of the LL$^{14}$C method is that it can be used to determine the partitioning of methane-carbon between CO$_2$ and cell biomass (the $^3$H method cannot accomplish this). Carbon (C) partitioning provides valuable data for C-cycle studies and for establishing C budgets. The HL$^{14}$C method can also provide C-partitioning data, its sample processing time lags between the $^3$H and LL$^{14}$C methods (due to filtering and CO$_2$ collection steps), it is costwise similar to the $^3$H method, but it requires a radiation van and has the greatest potential for priming samples. Thus, its most practical use is in high-CH$_4$ waters where C partitioning is of interest and radiation vans are available.

The rate measurements reported here are the first available from the ETNP region and include some of the lowest reported for the ocean. In the subsequent discussion of ETNP CH$_4$ dynamics, we will assume the parallel $^3$H and LL$^{14}$C rate data bracket the actual range of MO$_x$ rates, with the LL$^{14}$C as the minimum (underestimate due to loss of biomass through large pore size filters) and the $^3$H data as the maximum (overestimate due to priming and method backgrounds). While this leads to large ranges that are especially noticeable when rates are integrated to a regional scale, it also highlights the large uncertainties in our current understanding of ocean CH$_4$ budgets.

4.2. ETNP Environmental Controls on Methane and MO$_x$ Rates

Previous studies on CH$_4$ dynamics in the ETNP region have suggested that low O$_2$ concentrations limit MO$_x$ in the OMZ, thereby allowing coastal CH$_4$ to laterally advect long distances to the open ocean [Sansone et al., 2001, 2004]. Two aspects of our rate measurements support this idea. First, minima in MO$_x$ rates within the OMZ (Figure 5) show long turnover times: 2.8–33 years and 0.3–5.5 years for the LL$^{14}$C and $^3$H methods, respectively. Second, average turnover times ($\tau$, equation (1)) for OMZ waters (200–760 m) are 12 years (LL$^{14}$C) or 1.2 years ($^3$H) and are longer than the average 4.5 years (LL$^{14}$C) and 0.3 years ($^3$H) values in surface waters (5–200 m). Further evidence supporting offshore advection of CH$_4$ within the OMZ can be found in the relationship between CH$_4$ concentration and water density (Figure 7): the midwater CH$_4$ maximum at Stations 1, 4a, and 8, are all near the 26.8 kg m$^{-3}$ isopycnal. This suggests that CH$_4$ supplying the maximum is advected on a common density surface, possibly from organic-rich coastal sediments in contact with the OMZ.

Our LL$^{14}$C and $^3$H rate data show relationships with a number of environmental parameters and provide insight into the controls on MO$_x$ rates in ETNP waters. LL$^{14}$C fractional turnover rates ($k$) show weak, but statistically significant ($p < 0.05$) linear correlations with the product of CH$_4$ and O$_2$ concentrations (CH$_4$×O$_2$), O$_2$, temperature, salinity, and density (Table 3). $^3$H values of $k$ show statistically significant, although weaker, linear correlations with O$_2$, temperature, salinity, and density (Table 3). The strongest linear correlation appears between the LL$^{14}$C values of $k$ and CH$_4$×O$_2$ ($r^2 = 0.67$, df = 43, Figure 8 and Table 3). This suggests that the combined availability of CH$_4$ and O$_2$ (i.e., second-order kinetic control) may influence MO$_x$ rates in the ETNP region.
Both CH₄ and O₂ concentrations must be limiting (i.e., close to their respective half-saturation constants) for second-order kinetic control. Methane is likely limiting in all of our samples because it ranges from 0.27 to 19 nmol L⁻¹ and the CH₄ half-saturation constants reported for marine methanotrophs are ≥60 nmol L⁻¹ [Ward and Kilpatrick, 1990]. In contrast, O₂ is likely not a limiting factor in the majority of our samples, because only samples from the core of the OMZ (Figures 2 and 3) approach the 3.2 mmol L⁻¹ O₂ half-saturation constant [Devol, 1978]. Further, our samples originate from a number of different locations and depths in the ETNP region, which likely host methanotroph communities that vary in number, activity, and kinetic properties. Thus, systematic experimentation with the same methanotroph community (i.e., from the same location and depth) is needed to further examine the possibility of second-order kinetic control.

Generally, longer ³H and LL ¹⁴C turnover times (τ, the reciprocal of k, equation (2)) occur at colder water temperatures, lower CH₄ concentrations, and higher salinities and densities, while faster turnover rates are found at warmer temperatures, higher CH₄ and lower salinities and densities (Figure 9a–9h). This relationship with CH₄ has been reported with previous ³H rate measurements [Valentine et al., 2001; Mau et al., 2012] and may indicate the existence of less active methanotroph communities at lower CH₄ levels. The trend with temperature, which is an important factor in all metabolic processes [Gillooly et al., 2001], shows the expected Arrhenius pattern of turnover times increasing as temperatures drop. MO₄ rates were not used to investigate environmental correlations because rate calculations incorporate in situ CH₄ concentrations (equation (1)) and thus mathematically correlate MO₄ rates with CH₄ concentration.

### 4.3. Regional MO₄ Within the ETNP OMZ

Depth-integrated MO₄ rates can provide regional estimates of CH₄ oxidation. In order to examine CH₄ consumption within the OMZ, where the ETNP’s large CH₄ pool resides, MO₄ rates were depth integrated from 200 to 760 m and multiplied by the ETNP area (5.2 × 10⁶ km²) [Sansone et al., 2001]. These calculations yield regional sink estimates of 4.5–6.1 × 10¹⁰ and 26–100 × 10¹⁰ g CH₄ yr⁻¹ for the LL ¹⁴C and ³H methods, respectively, and show that substantial methanotrophic activity in the OMZ consumes CH₄ at depth, thereby preventing its eventual release to the atmosphere. MO₄ in the OMZ over the ETNP area consumes more CH₄ than that reported for the Eel River Basin (2 × 10⁶ g CH₄ yr⁻¹) [Valentine et al., 2001] and the Juan de Fuca Ridge hydrothermal plume (1.3 × 10⁶ g CH₄ yr⁻¹) [de Angelis et al., 1993], regions that are actually venting CH₄, but cover much smaller areas (25 km² and 170 km², respectively). These data show that slower MO₄ rates can indeed integrate to a significant CH₄ sink if functioning throughout a large water volume.

**Table 3. Summary of Linear Regression Analyses Between LL ¹⁴C or ³H Fractional Turnover Rate (k) and Environmental Parameters (Degrees of Freedom, df = 43)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LL ¹⁴C k (d⁻¹) Slope × 10⁻⁵</th>
<th>r²</th>
<th>p</th>
<th>³H k (d⁻¹) Slope × 10⁻⁵</th>
<th>r²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₄ × O₂ (nmol L⁻¹ × μmol L⁻¹)</td>
<td>0.13 × 10⁻⁵</td>
<td>0.67</td>
<td>&lt;0.0001</td>
<td>0.8 × 10⁻⁵</td>
<td>0.11</td>
<td>0.026</td>
</tr>
<tr>
<td>CH₄ (nmol L⁻¹)</td>
<td>2.0 × 10⁻⁵</td>
<td>0.00</td>
<td>0.84</td>
<td>-7.8 × 10⁻⁵</td>
<td>0.02</td>
<td>0.37</td>
</tr>
<tr>
<td>O₂ (μmol L⁻¹)</td>
<td>0.46 × 10⁻⁵</td>
<td>0.54</td>
<td>&lt;0.0001</td>
<td>4.3 × 10⁻⁵</td>
<td>0.22</td>
<td>0.0011</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>4.3 × 10⁻⁵</td>
<td>0.54</td>
<td>&lt;0.0001</td>
<td>66 × 10⁻⁵</td>
<td>0.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Salinity</td>
<td>-70 × 10⁻⁵</td>
<td>0.49</td>
<td>&lt;0.0001</td>
<td>-540 × 10⁻⁵</td>
<td>0.25</td>
<td>0.0005</td>
</tr>
<tr>
<td>Density (sigma-theta, kg m⁻³)</td>
<td>-17 × 10⁻⁵</td>
<td>0.61</td>
<td>&lt;0.0001</td>
<td>-210 × 10⁻⁵</td>
<td>0.32</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*The coefficient of determination for each linear regression.

*P value to evaluate the statistical significance of each linear regression.

**Figure 8.** Plot of LL ¹⁴C fractional turnover rates (k) versus the product of methane and oxygen concentrations (CH₄ × O₂).
Methane in the deeper section of the OMZ (400–760 m) is thought to have advected from organic-rich coastal sediments to the open ocean. Depth-integrated \( \text{MO}_x \) rates scaled to the ETNP area show that 0.39–0.44 \( \times 10^{11} \) g CH\(_4\) yr\(^{-1}\) (LL\(^{14}\)C) or 0.56–2.6 \( \times 10^{11} \) g CH\(_4\) yr\(^{-1}\) (\(^3\)H) are consumed in this interval of the OMZ. We expect that the amount of CH\(_4\) contributed from coastal sediments to this depth interval is similar to the amount of CH\(_4\) consumed within the interval. This assumes that CH\(_4\) concentrations are not changing with time and that the rate of CH\(_4\) transport out of the OMZ is small compared to \( \text{MO}_x \) rates.

We scaled up two coastal margin flux measurements to estimate the amount of CH\(_4\) diffusing from coastal sediments (0.24 and 1.7 \( \mu \)mol m\(^{-2}\) d\(^{-1}\), open margin sites with water depths in the 400–760 m range from Sansone et al. [2004]. Data from Google Earth were used to estimate that a ~17,800 km\(^2\) area of coastal sediment could potentially supply CH\(_4\) to the 400–760 m OMZ waters through diffusion (see Text S2 and Figure S1 in the supporting information). Combining the diffusive fluxes and sediment area yields a source estimate of 0.25–1.8 \( \times 10^{8} \) g CH\(_4\) yr\(^{-1}\), which is 10\(^3\) smaller than the \( \text{MO}_x \) sink estimate for the 400–760 m interval. Such a discrepancy suggests the presence of other unidentified CH\(_4\) sources in the region (e.g., areas of greater diffusive flux or ebullition, decomposing methane hydrates) and provides an upper constraint for such sources. Even though the 10\(^3\) difference is large and therefore indicates other sources, the data used for the source and sink estimates is sparse compared to the whole area of the ETNP; thus, we cannot rule out the possibility that the difference is within the error of our calculation.

### 4.4. \( \text{MO}_x \) in ETNP Surface Waters

The subsurface CH\(_4\) maximum contributes 25% of the ocean’s estimated CH\(_4\) source to the atmosphere [Reeburgh, 2007]. The maximum is thought to be comprised of CH\(_4\) produced locally by methanogens in anaerobic microenvironments or by the biological decomposition of methylphosphonates [Reeburgh, 2007]. \( \text{MO}_x \) may play a role in mitigating the eventual release of this CH\(_4\) to the atmosphere (e.g., Nihous and Masutani [2006]), but previous studies have suggested that sea-air flux is the main sink for CH\(_4\) in open ocean surface waters and that \( \text{MO}_x \) is not significant [Jones, 1991; Holmes et al., 2000]. Our depth-integrated \( \text{MO}_x \) rates (~0–200 m, through the subsurface maximum) show that 0.25–1.3 and 17–47 \( \mu \)mol CH\(_4\) m\(^{-2}\) d\(^{-1}\) for the LL\(^{14}\)C and \(^3\)H methods, respectively, are consumed in ETNP surface waters (Table 4). Whereas, sea-air fluxes of CH\(_4\) from surface waters, estimated using the techniques outlined in Holmes et al. [2000], ranged from ~0.64 to 9.4 \( \mu \)mol CH\(_4\) m\(^{-2}\) d\(^{-1}\) (Table 4). The negative flux values at Stations 5 and 6 indicate a CH\(_4\) flux into surface waters, reflecting efficient \( \text{MO}_x \) that consumes upward fluxes of CH\(_4\) before they reach the atmosphere, as well as some amount of atmospheric CH\(_4\). At stations with a positive sea-air flux, summing the integrated \( \text{MO}_x \) rates and sea-air flux values gives an estimate of total CH\(_4\) losses in ETNP surface waters and shows that \( \text{MO}_x \) on average makes up 25% (LL\(^{14}\)C) or 85% (\(^3\)H) of these losses. The results, from both the LL\(^{14}\)C and \(^3\)H methods, show that methanotrophy is a significant sink for CH\(_4\) in ETNP surface waters and that it mitigates the release of CH\(_4\) from the subsurface maximum to the atmosphere.

### 5. Conclusions

The first \( \text{MO}_x \) rates reported for the ETNP (1) support the idea that the high CH\(_4\) concentrations in the OMZ come from coastal sediments and persist due to low \( \text{MO}_x \) rates and (2) show that water column \( \text{MO}_x \) rates,
Although slow, still provide a significant internal CH₄ sink that limits the flux of CH₄ from ocean to atmosphere. Future improvements in rate measurement methods, especially if they confirm the ability to measure very low rates with the LL¹⁴C tracer, will further improve our understanding of the role of MOx in CH₄ ocean-atmosphere fluxes and how they may change in the future when OMZs are expected to expand. Several improvements to both the ³H and LL¹⁴C methods can be recommended for future MOx rate measurements. The ³H method background can be better quantified using killed control samples as method blanks (either to subtract from rate samples or set a detection limit [e.g., Mendes et al., 2015]). For this, it is important to treat the killed controls in such a manner that the microbial activity is arrested well before the tracer is added. For the LL¹⁴C method, filters with a pore size of 0.2 μm should be used for capturing the bacterial biomass. For both methods, if a Q₁₀ correction is to be applied to compensate for incubation temperatures, the sample temperatures need to be limited to a narrow range and be continuously monitored over the incubation to minimize the error associated with a Q₁₀ correction. These easily implement updates that should improve ³H and LL¹⁴C parallel rate measurements; however, highly controlled parallel ³H and LL¹⁴C rate experiments are required to identify the underlying cause(s) of any discrepancies in parallel rates. Overall, more work is needed to bring all three tracer-based rate measurements (³H, HL¹⁴C, and LL¹⁴C) in line, and accomplishing this is essential for establishing accurate CH₄ budgets in the ocean water column.

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