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## RESEARCH ARTICLE

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## Key Points:

- The first CH<sub>4</sub> oxidation (MO<sub>x</sub>) rates in the eastern tropical Pacific (ETP) Ocean
- Water column MO<sub>x</sub> strongly mitigates sea-air CH<sub>4</sub> flux in the ETP region
- Advances in rate measurements are needed to provide accurate ocean CH<sub>4</sub> budgets

## Supporting Information:

- Supporting Information S1

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## Methane oxidation in the eastern tropical North Pacific Ocean water column

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**Abstract** We report methane (CH<sub>4</sub>) concentration and methane oxidation (MO<sub>x</sub>) rate measurements from the eastern tropical north Pacific (ETNP) water column. This region comprises low-CH<sub>4</sub> waters and a depth interval (~200–760 m) of CH<sub>4</sub> supersaturation that is located within a regional oxygen minimum zone (OMZ). MO<sub>x</sub> rate measurements were made in parallel using tracer-based methods with low-level <sup>14</sup>C-CH<sub>4</sub> (LL <sup>14</sup>C) and <sup>3</sup>H-CH<sub>4</sub> (<sup>3</sup>H). The two tracers showed similar trends in MO<sub>x</sub> rate with water depth, but consistent with previous work, the LL <sup>14</sup>C rates (range: 0.034–15 × 10<sup>-3</sup> nmol CH<sub>4</sub> L<sup>-1</sup> d<sup>-1</sup>) were systematically slower than the parallel <sup>3</sup>H rates (range: 0.098–4000 × 10<sup>-3</sup> nmol CH<sub>4</sub> L<sup>-1</sup> d<sup>-1</sup>). Priming and background effects associated with the <sup>3</sup>H-CH<sub>4</sub> tracer and LL <sup>14</sup>C filtering effects are implicated as the cause of the systematic difference. The MO<sub>x</sub> 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 CH<sub>4</sub> turnover within the OMZ that may permit CH<sub>4</sub> derived from coastal sediments to travel great lateral distances. The MO<sub>x</sub> rate constants correlate with both CH<sub>4</sub> and oxygen concentrations, suggesting that their combined availability regulates MO<sub>x</sub> rates in the region. Depth-integrated MO<sub>x</sub> rates provide an upper limit on the magnitude of regional CH<sub>4</sub> sources and demonstrate the importance of water column MO<sub>x</sub>, even at slow rates, as a sink for CH<sub>4</sub> that limits the ocean-atmosphere CH<sub>4</sub> flux in the ETNP region.

### 1. Introduction

Methane (CH<sub>4</sub>) is a powerful greenhouse gas that plays important roles in atmospheric chemistry [Cicerone and Oremland, 1988]. The marine environment constitutes a vast reservoir of CH<sub>4</sub> (>10<sup>19</sup> g carbon) [Dickens, 2003; Zhang et al., 2011], but due to highly efficient and microbially mediated CH<sub>4</sub> oxidation, very little marine CH<sub>4</sub> makes its way to the atmosphere. Current estimates indicate that only 4–15 Tg CH<sub>4</sub> yr<sup>-1</sup>, or 0.7–2.7% of the annual total sources of atmospheric CH<sub>4</sub>, 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 CH<sub>4</sub> oxidation together nearly balance CH<sub>4</sub> production in the marine environment and act as a “cap” that limits the influence of this large CH<sub>4</sub> reservoir on the atmosphere [Reeburgh, 2007]. However, many aspects of the marine CH<sub>4</sub> budget are not well understood or quantified. For example, the estimates of total CH<sub>4</sub> oxidation in ocean waters and shallow sediments that are used to constrain marine sources range between 75 and 304 Tg CH<sub>4</sub> yr<sup>-1</sup> [Hinrichs and Boetius, 2002; Reeburgh, 2007]. Furthermore, the effects of environmental factors that can regulate the rate of CH<sub>4</sub> oxidation, including nutrient availability, temporal variations in CH<sub>4</sub> 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 CH<sub>4</sub>.

Few measurements of CH<sub>4</sub> oxidation rates exist for oxic ocean waters (MO<sub>x</sub>), and the overall consumption of marine CH<sub>4</sub> by this process is less studied than anaerobic CH<sub>4</sub> oxidation [Reeburgh, 2007]. The available MO<sub>x</sub> rate measurements span several orders of magnitude (~0.001–10 nmol CH<sub>4</sub> L<sup>-1</sup> d<sup>-1</sup>) and are insufficient to reveal the environmental factors that regulate MO<sub>x</sub> [Mau et al., 2013]. An especially important, yet unresolved issue is whether oxidation ceases when CH<sub>4</sub> 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-CH<sub>4</sub> concentrations (<1–5 nmol L<sup>-1</sup>), and MO<sub>x</sub> 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 CH<sub>4</sub> concentrations [Scranton and Brewer, 1978; Rehder et al., 1999; Heeschen et al., 2004; Keir et al., 2005]. These are quite long compared to turnover times of 5–15 days measured in waters where active CH<sub>4</sub> seepage occurs [de Angelis et al., 1993; Pack et al., 2011; Heintz et al., 2012]. The large volume of low-CH<sub>4</sub> waters in the central and deep ocean means that even slow MO<sub>x</sub> rates in these waters can provide an important sink in the overall marine CH<sub>4</sub> budget.

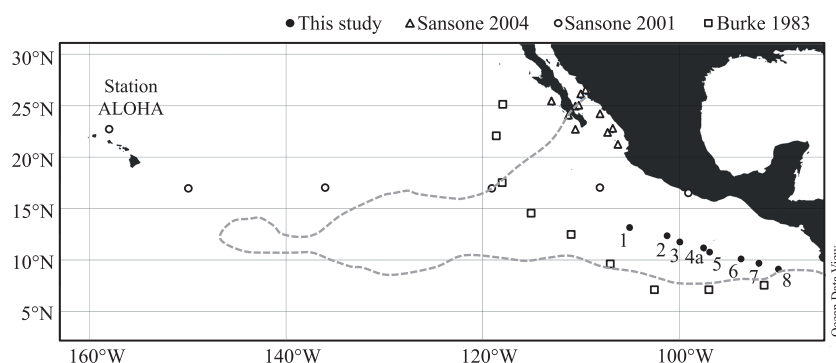
One reason for the lack of MO<sub>x</sub> rate measurements in oxic ocean waters is the challenge of measuring slow rates at low-CH<sub>4</sub> concentrations. Methanotrophs, bacteria capable of using CH<sub>4</sub> as their sole source of carbon and energy, oxidize CH<sub>4</sub> in oxic waters via the net reaction:



MO<sub>x</sub> rates are measured by adding <sup>14</sup>C-CH<sub>4</sub> or <sup>3</sup>H-CH<sub>4</sub> tracer to water samples, incubating the samples for a given period of time, then removing the unreacted CH<sub>4</sub> and quantifying the amount of tracer that accumulated in the products. Well-established tracer-based methods for the direct measurement of MO<sub>x</sub> rates use <sup>14</sup>C-CH<sub>4</sub> or <sup>3</sup>H-CH<sub>4</sub> tracer at high levels (abbreviated as HL <sup>14</sup>C and <sup>3</sup>H); they use 10<sup>5</sup> Bq per 160 mL of water and measure the conversion of the CH<sub>4</sub> tracer to carbon dioxide (CO<sub>2</sub>) and cell biomass (HL <sup>14</sup>C tracer) or water (<sup>3</sup>H 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 CH<sub>4</sub> 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 CH<sub>4</sub> concentrations by ~2–10 nmol L<sup>-1</sup> for <sup>3</sup>H, and ~400 nmol L<sup>-1</sup> for HL <sup>14</sup>C [Mau et al., 2013; Valentine et al., 2001]. In the open and deep ocean regions where CH<sub>4</sub> concentrations are 5 nmol L<sup>-1</sup> or lower, these levels of tracer addition raise the amount of CH<sub>4</sub> significantly (>100 times for HL <sup>14</sup>C), possibly accelerating the measured MO<sub>x</sub> rates through priming [Ward et al., 1987]. Further, the interaction of the <sup>3</sup>H-CH<sub>4</sub> radioactive decay (which has a 12.3 years half-life compared to 5730 years for <sup>14</sup>C-CH<sub>4</sub>) with surrounding molecules and <sup>3</sup>H-CH<sub>4</sub> isotope exchange (a process not observed with <sup>14</sup>C-CH<sub>4</sub> tracers) can lead to high <sup>3</sup>H method backgrounds. These backgrounds are not well quantified and may become significant at slow MO<sub>x</sub> rates. A recently developed low-level <sup>14</sup>C-CH<sub>4</sub> method (LL <sup>14</sup>C) [Pack et al., 2011] that raises CH<sub>4</sub> concentrations in samples by only 0.004 nmol L<sup>-1</sup>, uses ~10<sup>6</sup> times less radioactivity and accelerator mass spectrometry (AMS) to measure radioactivity, addresses the challenge of MO<sub>x</sub> rate measurements in low-CH<sub>4</sub> waters (background seawater by AMS is on the order of 0.001 Bq per 160 mL). A previous comparison of the <sup>3</sup>H and LL <sup>14</sup>C methods in coastal waters demonstrated good agreement at rates faster than 0.1 nmol CH<sub>4</sub> L<sup>-1</sup> d<sup>-1</sup>, but at slower rates the LL <sup>14</sup>C results were systematically lower than the <sup>3</sup>H results [Pack et al., 2011]. Additional LL <sup>14</sup>C and <sup>3</sup>H comparisons along with controlled experiments are needed to continue establishing the LL <sup>14</sup>C method.

In this study, we applied the LL <sup>14</sup>C and <sup>3</sup>H tracer methods to measure MO<sub>x</sub> rates in the low-CH<sub>4</sub> 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 × 10<sup>6</sup> km<sup>2</sup> (Figure 1) [Sansone et al., 2001]. Methane concentrations in the OMZ are 79 nmol L<sup>-1</sup> at the Mexican coast and >10 nmol L<sup>-1</sup> hundreds of kilometers off the coast [Sansone et al., 2001, 2004]. Overall, the ETNP comprises the largest reported open ocean CH<sub>4</sub> pool (~0.3 Tg CH<sub>4</sub>) and the OMZ provides a local maximum in CH<sub>4</sub> compared to open ocean surface waters of ~2–8 nmol L<sup>-1</sup> and deep ocean background waters of ~2–3 nmol L<sup>-1</sup> [Sansone et al., 2001].

Past studies of CH<sub>4</sub> dynamics in the ETNP region indicate that CH<sub>4</sub> found within the OMZ has two probable sources: CH<sub>4</sub> produced in situ and CH<sub>4</sub> advected from coastal sediments. Methane in the upper 200–400 m has depleted δ<sup>13</sup>C-CH<sub>4</sub> values (–45‰ to –42‰) that point to a lightly oxidized biogenic source [Sansone et al., 2001]. Hence, it is thought that shallow CH<sub>4</sub> 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;



**Figure 1.** Map of the ETNP (eastern tropical north Pacific) region produced via Ocean Data View software (Schlitzer [2010]) and showing the stations occupied for this study (closed circles, numbered 1–8) and the location of past studies on methane dynamics in the region. The previous studies include Sansone *et al.* [2004] (open triangles), Sansone *et al.* [2001] (open circles), and Burke *et al.* [1983] (open squares). The dashed gray line is the OMZ area defined by Sansone *et al.* [2001] where the oxygen is  $9 \mu\text{mol L}^{-1}$  at 400 m. Station ALOHA is the location of ongoing time series experiments [Karl, 1999].

Karl *et al.*, 2008]. Methane below 400 m is enriched in  $^{13}\text{C}$  ( $-26\text{‰}$  to  $-30\text{‰}$ ) near the coast and becomes even more enriched with distance from the coast ( $10\text{--}15\text{‰}$ ) [Holmes *et al.*, 2000; Sansone *et al.*, 2001]. Microbial  $\text{CH}_4$  oxidation preferentially uses  $^{12}\text{C}\text{-CH}_4$  and leaves the residual  $\text{CH}_4$  enriched in  $^{13}\text{C}$  [Coleman *et al.*, 1981]. Thus, the deeper pool is believed to be  $\text{CH}_4$  that was moderately oxidized within the OMZ while advecting from organic-rich coastal sediments to the open ocean ( $>1500$  km). The low  $\text{O}_2$  conditions in the OMZ are hypothesized to slow  $\text{MO}_x$  and allow  $\text{CH}_4$  to travel long distances [Sansone *et al.*, 2001, 2004].

While a number of studies have focused on  $\text{CH}_4$  dynamics in the ETNP region [Burke *et al.*, 1983; Holmes *et al.*, 2000; Sansone *et al.*, 2001, 2004], to date no direct measurements of  $\text{MO}_x$  rates have been reported and the hypotheses those studies generated about low  $\text{MO}_x$  in the OMZ or how  $\text{MO}_x$  affects the potential flux of  $\text{CH}_4$  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  $\text{CH}_4$  dynamics in oxygen minimum zones will be essential in predicting potential future changes in  $\text{CH}_4$  pool size and  $\text{CH}_4$  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  $\text{CH}_4$  concentration and  $^3\text{H}\text{-CH}_4$  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  $^{14}\text{C}\text{-CH}_4$  oxidation rate measurements. Samples were taken directly from the Niskin

**Table 1.** Stations Occupied in the ETNP Region During This Study

Station	Latitude ( $^{\circ}\text{N}$ )	Longitude ( $^{\circ}\text{W}$ )
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.  $\text{MO}_x$  rate measurements were made at all stations occupied using the  $^3\text{H-CH}_4$  tracer but only at Stations 1, 3, 6, and 8 (Figure 1) with the LL  $^{14}\text{C-CH}_4$  tracer, because the method required more processing time per sample.

## 2.2. Analytical Methods

### 2.2.1. Oxygen and Methane Concentrations

Measurements of  $\text{O}_2$  concentration were made with an SBE 43 sensor on the CTD package and verified with Winkler titrations throughout the cruise. After collection, samples for  $\text{CH}_4$  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  $\text{N}_2$ ) 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  $\text{CH}_4$  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  $\text{CH}_4$  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  $\pm 11\%$ . Air-equilibrated  $\text{CH}_4$  solubility was estimated using the Yamamoto *et al.* [1976] Bunsen coefficients based on in situ salinity and potential temperature and an atmospheric  $\text{CH}_4$  mixing ratio of  $1.8 \mu\text{L L}^{-1}$ . Percent saturation was calculated by comparing the measured  $\text{CH}_4$  concentrations to the air-equilibrated concentrations.

### 2.2.2. $^3\text{H-CH}_4$ Oxidation Rate Measurements

$\text{MO}_x$  rate measurements with the  $^3\text{H-CH}_4$  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  $\mu\text{L}$  aliquots of  $^3\text{H-CH}_4$  (370 kBq, 6360 kBq  $\text{mg}^{-1}$  tracer, 4  $\mu\text{mol L}^{-1}$   $\text{CH}_4$  in  $\text{N}_2$ ; 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  $\text{N}_2$  for 30 min to remove unreacted  $\text{CH}_4$ . Finally, samples were resealed and stored for transport back to shore.

In a shore-based laboratory, the quantity of  $^3\text{H-H}_2\text{O}$  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  $^3\text{H-CH}_4$  tracer that was converted to  $^3\text{H-H}_2\text{O}$  by the incubation time.  $\text{MO}_x$  rates ( $R_{\text{oxd}}$ ) were calculated as the product of  $k$  and the in situ  $\text{CH}_4$  concentration ( $[\text{CH}_4]$ ) assuming first-order kinetics:

$$R_{\text{oxd}} = k \times [\text{CH}_4] \quad (1)$$

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

$$\tau = 1/k \quad (2)$$

Killed controls were taken (1–2 per CTD cast) to spot check that nonbiological processes did not incorporate the  $^3\text{H-CH}_4$  tracer into the aqueous phase and to test for impurities in the  $^3\text{H-CH}_4$  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  $^3\text{H-CH}_4$  and then subjected to the procedures outlined above.

### 2.2.3. Low-Level $^{14}\text{C-CH}_4$ Oxidation Rate Measurements

*Preparation and Activity of the LL  $^{14}\text{C-CH}_4$  Tracer and  $^{14}\text{C-Free CO}_2$ .* A diluted form of the  $^{14}\text{C-CH}_4$  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  $^{14}\text{C-free CO}_2$  (Matheson Gas, anaerobic grade) in a preevacuated 6 L stainless canister. The activity of the resulting  $^{14}\text{C-CH}_4$  tracer was determined using the methods outlined by Pack *et al.* [2011] and found to be  $0.00013 \pm 0.00001$  kBq per 50  $\mu\text{L}$  of tracer ( $5.4 \pm 0.4 \times 10^{-14}$  moles  $^{14}\text{C}$  per 50  $\mu\text{L}$  tracer or  $0.00139 \pm 0.00009$  kBq  $\text{mg}^{-1}$  tracer). The  $^{14}\text{C-free CO}_2$  used with background samples was prepared by adding the same  $\text{CO}_2$  as used with the tracer (Matheson Gas, anaerobic grade) to a preevacuated 2 L stainless canister.

*Shipboard  $^{14}\text{C}$  Labeling.* Samples for LL  $^{14}\text{C}$  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  $^3\text{H}$  measurements. The equipment used with the  $^3\text{H}$  rate samples had previously been exposed to HL  $^{14}\text{C}$  tracers, so in order to avoid contamination of the LL  $^{14}\text{C}$  samples with residual HL  $^{14}\text{C}$  tracer, it was important to completely separate the processing of the  $^3\text{H}$  and LL  $^{14}\text{C}$  samples. One of the two 120 mL water samples collected from each depth was injected with 50  $\mu\text{L}$  of  $^{14}\text{C}\text{-CH}_4$  (0.00013 kBq, 0.00139 kBq  $\text{mg}^{-1}$  tracer, 0.0086  $\mu\text{mol L}^{-1}$   $\text{CH}_4$  in  $^{14}\text{C}$ -free  $\text{CO}_2$ ), while the other was treated with 50  $\mu\text{L}$  of pure  $^{14}\text{C}$ -free  $\text{CO}_2$ . This treatment yielded sets of labeled and background samples. Following injection, samples were shaken to facilitate  $\text{CH}_4$  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  $\text{CH}_4$ . For filtering, we used procedures that collected the cell biomass without exposing sample filtrate to atmospheric  $\text{CO}_2$ , which can alter the sample  $^{14}\text{C}\text{-CO}_2$  content. First, 60 mL of sample was transferred to a syringe by displacement with an equivalent amount of UHP  $\text{N}_2$ . Next, a syringe filter holder (EMD Millipore Corporation No. SX0002500) with a 1.2  $\mu\text{m}$  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  $\text{N}_2$  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  $^{14}\text{C}\text{-CO}_2$  gas oxidation product as carbonate ions ( $\text{CO}_3^{2-}$ ) in solution. The unreacted  $\text{CH}_4$  was then removed from the sample filtrate by purging with UHP  $\text{N}_2$  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  $\text{N}_2$ , 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  $\text{N}_2$ . After purging was complete, the gray butyl sample stoppers were replaced with blue butyl stoppers (Bellco Glass No. 2048-11800) in a homemade glove chamber with UHP  $\text{N}_2$  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  $-10^\circ\text{C}$  until  $^{14}\text{C}\text{-AMS}$  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  $^{14}\text{C}\text{-CH}_4$  injection, incubated, and treated as described above.

*Shore-Based  $^{14}\text{C}\text{-AMS}$  Analyses.* Shore-based measurements quantified the  $^{14}\text{C}$  in the dissolved inorganic carbon (DIC:  $\text{CO}_2$ , carbonic acid, bicarbonate, and carbonate) and cell biomass that accumulated in samples during incubation with the LL  $^{14}\text{C}\text{-CH}_4$  tracer. DIC was extracted from  $^{14}\text{C}$ -labeled background and killed control samples and the  $^{14}\text{C}$ -content measured at the University of California, Irvine Keck Carbon Cycle AMS facility as described in Pack *et al.* [2011]. However, our  $^{14}\text{C}$ -labeled samples did not require dilution with  $^{14}\text{C}$ -free  $\text{CO}_2$  prior to analysis because they were well below the maximum AMS detection limit ( $\sim 8$  times modern under the standard operating conditions at the Keck Carbon Cycle AMS facility). Procedure blanks were determined from a  $^{14}\text{C}$ -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  $^{14}\text{C}\text{-AMS}$  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 acetanilide, 80 mg cupric oxide, and silver wire (1 mm in diameter,  $\sim 3$  mm long). Acetanilide was used as a dead carbon ( $^{14}\text{C}$ -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  $^{14}\text{C}$  and  $^3\text{H}$  Rate Samples

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

<sup>a</sup>The number of rate samples that are within the listed depth range.

combusted at 900°C for 2 h. This process quantitatively converted the sample cell biomass and acetanilide to  $\text{CO}_2$ , and the  $\text{CO}_2$  was prepared for  $^{14}\text{C}$ -AMS analysis following the methods outlined in Xu *et al.* [2007]. The  $^{14}\text{C}$  values of filters from background samples were averaged and used as a procedure blank for filters with  $^{14}\text{C}$ -labeled cell material.

**Rate Calculations.** The  $^{14}\text{C}$  that accumulated in the DIC and cell biomass ( $^{14}\text{C}_{\Delta\text{DIC}}$  and  $^{14}\text{C}_{\Delta\text{CM}}$ , respectively) during incubation with LL  $^{14}\text{C}$ - $\text{CH}_4$  tracer was calculated as follows.

$$^{14}\text{C}_{\Delta\text{DIC}} = (^{14}\text{C}_{\text{LDIC}} - ^{14}\text{C}_{\text{BDIC}}) \times \text{DIC} \times V_s \quad (3)$$

$$^{14}\text{C}_{\Delta\text{CM}} = (^{14}\text{C}_{\text{LCM}} - ^{14}\text{C}_{\text{BCM avg}}) \times \text{CO}_2 \times V_s/V_f \quad (4)$$

Here  $^{14}\text{C}_{\text{LDIC}}$  and  $^{14}\text{C}_{\text{BDIC}}$  are the  $^{14}\text{C}$ : $^{12}\text{C}$  ratios of the DIC in the labeled and background samples, respectively; DIC is the sample DIC concentration ( $\Sigma\text{CO}_2$ ); and  $V_s$  is the sample volume.  $V_f$  is the volume of sample filtered;  $\text{CO}_2$  is the moles of  $\text{CO}_2$  recovered from filter combustion with acetanilide; and  $^{14}\text{C}_{\text{LCM}}$  and  $^{14}\text{C}_{\text{BCM avg}}$  are the  $^{14}\text{C}$ : $^{12}\text{C}$  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  $^{14}\text{C}$ - $\text{CH}_4$  added to each sample ( $^{14}\text{CH}_4$ ):

$$k = \frac{(^{14}\text{C}_{\Delta\text{DIC}} + ^{14}\text{C}_{\Delta\text{CM}}) / ^{14}\text{CH}_4}{\text{incubation time}} \quad (5)$$

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

$$P_{\text{CL}} = 100 \times \frac{^{14}\text{C}_{\Delta\text{CM}}}{(^{14}\text{C}_{\Delta\text{DIC}} + ^{14}\text{C}_{\Delta\text{CM}})} \quad (6)$$

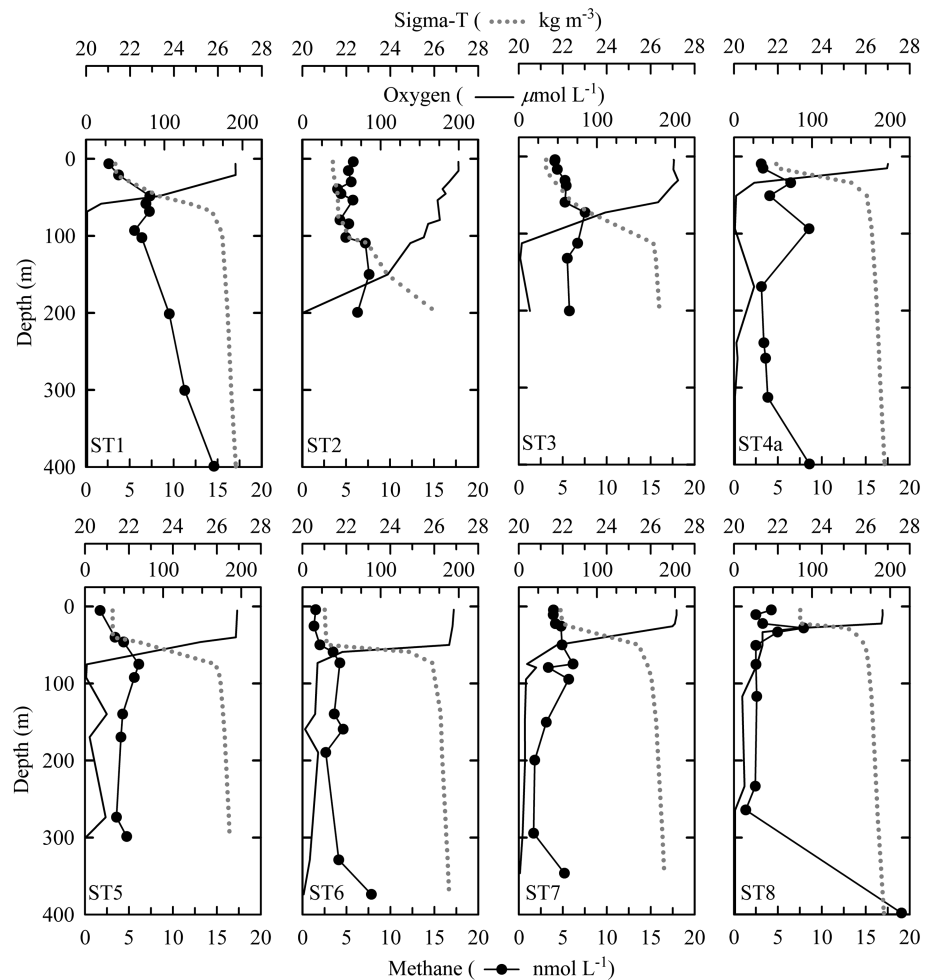
#### 2.2.4. $Q_{10}$ Correction for $\text{MO}_x$ Rates

Incubation temperature is an important factor in  $\text{MO}_x$  rate measurements [Heintz, 2011], and every effort was made to incubate parallel-processed LL  $^{14}\text{C}$  and  $^3\text{H}$  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  $^{14}\text{C}$  and  $^3\text{H}$  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  $^{14}\text{C}$  and  $^3\text{H}$  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  $^{14}\text{C}$  and  $^3\text{H}$  rate would be, had samples been incubated exactly at in situ temperatures (see equation (S1) and Text S1 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 $\text{MO}_x$ 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  $2 \mu\text{mol L}^{-1}$  by 300 m depth. The OMZ extended to ~750–800 m depth, with  $\text{O}_2$  levels increasing in deeper waters (Figure 3).

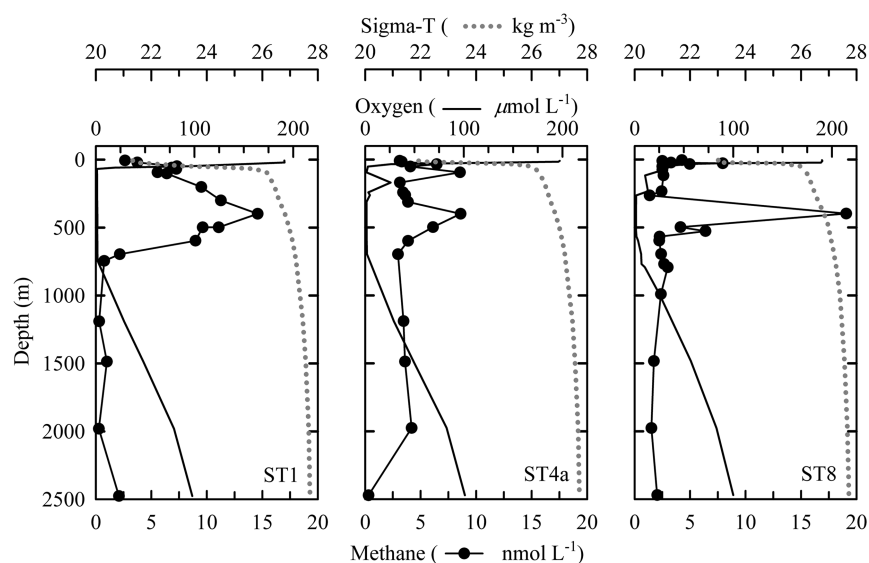


**Figure 2.** Profiles of methane, oxygen, and sigma-t in the upper 400 m of the water column for Stations 1–8.

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  $\text{CH}_4$  concentrations of 4.6–7.9  $\text{nmol L}^{-1}$  (189–367% supersaturated with respect to the atmosphere). The second feature was a midwater maximum centered in the OMZ with  $\text{CH}_4$  concentrations of 8.6 to 19  $\text{nmol L}^{-1}$  (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  $\text{CH}_4$  in the mixed layer and surface waters in some areas of the ETNP. Below the OMZ (>750–800 m),  $\text{CH}_4$  concentrations transitioned to undersaturated levels and reached a minimum value of 0.27  $\text{nmol L}^{-1}$  (8% saturated).

The  $\text{MO}_x$  rates in this study ranged from 0.000034 to 0.015  $\text{nmol CH}_4 \text{L}^{-1} \text{d}^{-1}$  for the LL  $^{14}\text{C}$  method and 0.000098–4.0  $\text{nmol CH}_4 \text{L}^{-1} \text{d}^{-1}$  for the  $^3\text{H}$  method. Profiles of  $\text{MO}_x$  rates (Figures 4 and 5) showed highest values near the subsurface  $\text{CH}_4$  maximum: the LL  $^{14}\text{C}$  rates increased to 0.0016–0.015  $\text{nmol CH}_4 \text{L}^{-1} \text{d}^{-1}$ , while the  $^3\text{H}$  rates reached 0.17–4.0  $\text{nmol CH}_4 \text{L}^{-1} \text{d}^{-1}$ . Higher  $\text{MO}_x$  rates were also present in the OMZ near the midwater  $\text{CH}_4$  maximum, with rates reaching 0.010–0.012  $\text{nmol CH}_4 \text{L}^{-1} \text{d}^{-1}$  and 0.048–0.17  $\text{nmol CH}_4 \text{L}^{-1} \text{d}^{-1}$  for the LL  $^{14}\text{C}$  and  $^3\text{H}$  methods, respectively. These higher  $\text{MO}_x$  rates were bounded, above and below, with lower  $\text{MO}_x$  rates that reached minimum values of 0.00022  $\text{nmol CH}_4 \text{L}^{-1} \text{d}^{-1}$  (LL  $^{14}\text{C}$ ) and 0.0019  $\text{nmol CH}_4 \text{L}^{-1} \text{d}^{-1}$  ( $^3\text{H}$ ). Below the OMZ,  $\text{MO}_x$  rates slowed to <0.00046  $\text{nmol CH}_4 \text{L}^{-1} \text{d}^{-1}$  (LL  $^{14}\text{C}$ ) and <0.12  $\text{nmol CH}_4 \text{L}^{-1} \text{d}^{-1}$  ( $^3\text{H}$ ).





**Figure 3.** Profiles of methane, oxygen, and sigma-t down to 2500 m in the water column for Stations 1, 4a, and 8.

The fraction of  $^{14}\text{C}\text{-CH}_4$  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  $^{14}\text{C}$  for the AMS analysis used here, and filters from samples with turnover times  $>700$  days did not contain enough  $^{14}\text{C}$ .

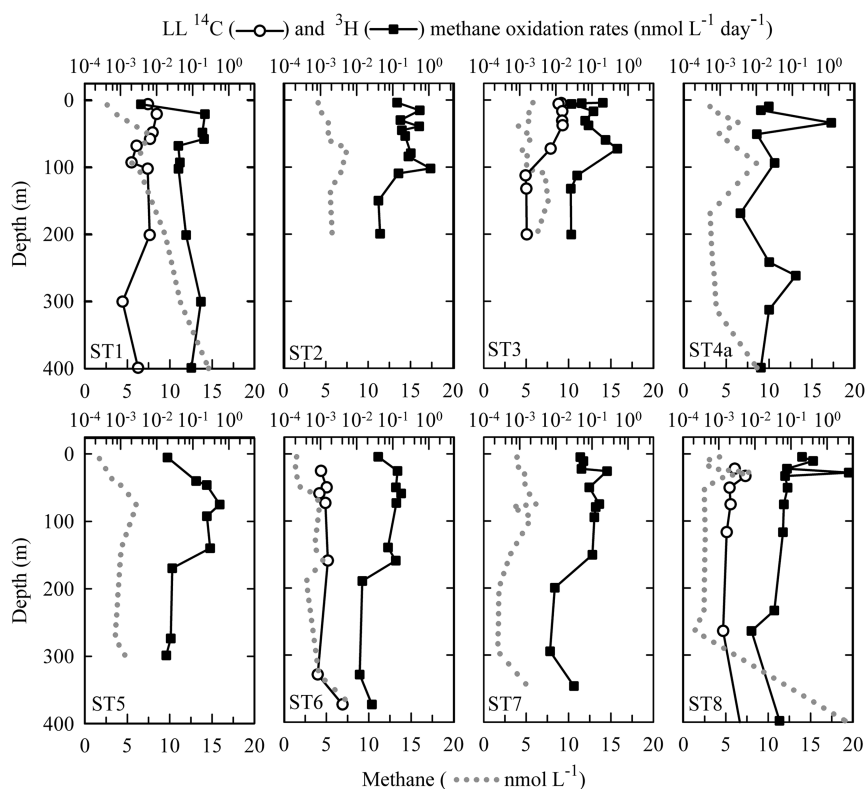
### 3.2. Comparison of the Low-Level $^{14}\text{C}\text{-CH}_4$ and $^3\text{H}\text{-CH}_4$ Rates

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  $^{14}\text{C}$  were systematically lower than those measured using  $^3\text{H}$  (Figures 4 and 5). Forty-four of the LL  $^{14}\text{C}$  rates were 2–184 (median: 16) times slower than the parallel  $^3\text{H}$  rates, while one LL  $^{14}\text{C}$  rate was 1.6 times faster (Figure 6).

### 3.3. Duplicate and Killed Control Rate Samples

The mean coefficient of variation from  $^3\text{H}$  rate measurements on 112 pairs of duplicate samples was 18% (range: 0.6–91%). For the LL  $^{14}\text{C}$  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,  $^{14}\text{C}\text{-AMS}$ , procedure blanks, sample volume, etc.) in error propagation equations. These analytic errors, withstanding the  $Q_{10}$  correction, were on average 17% for both the  $^3\text{H}$  and LL  $^{14}\text{C}$  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% ( $^3\text{H}$ ) and 59% (LL  $^{14}\text{C}$ ).

Killed control samples from the  $^3\text{H}$  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  $^{14}\text{C}$  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  $^{14}\text{C}\text{-CH}_4$  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  $^{14}\text{C}\text{-CH}_4$  was added and that NaOH in killed controls must be introduced well before the  $^{14}\text{C}\text{-CH}_4$  tracer. Abiotic uptake of  $^{14}\text{C}\text{-CH}_4$  tracer or impurities in the tracer could also cause high killed controls, but previous work with the same batch of  $^{14}\text{C}\text{-CH}_4$  tracer [Pack *et al.*, 2011] excludes this possibility.



**Figure 4.** Profiles of methane, and LL  $^{14}\text{C}$  and  $^3\text{H}$   $\text{MO}_x$  rates in the upper 400 m of the water column for Stations 1–8. The error bars for the LL  $^{14}\text{C}$  rates are combined analytic errors. The error bars on the  $^3\text{H}$  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.

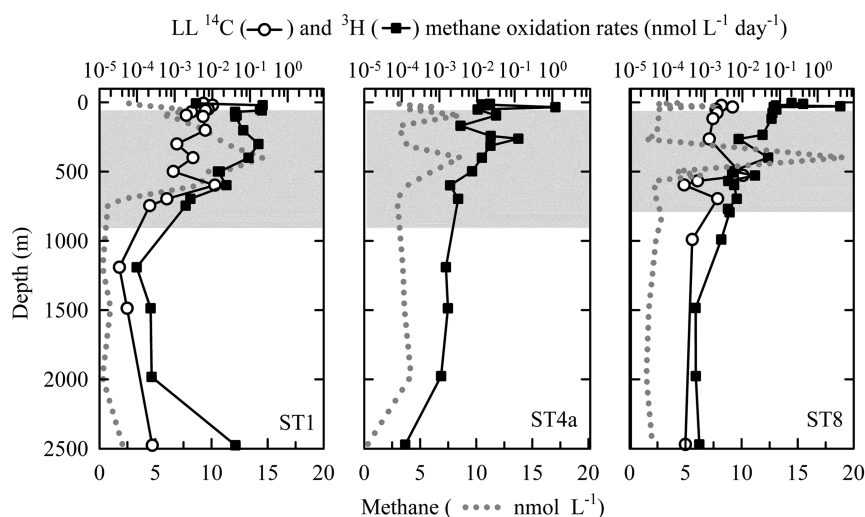
## 4. Discussion

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

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

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

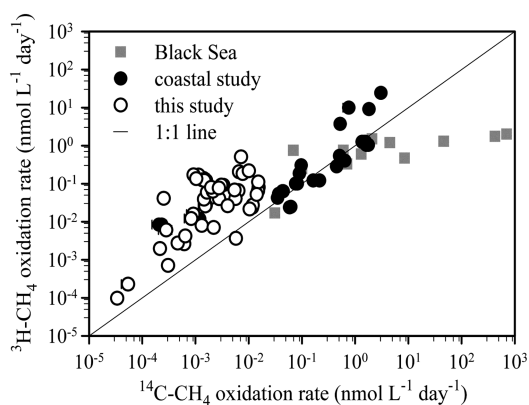
Priming and method backgrounds associated with the  $^3\text{H}$ - $\text{CH}_4$  tracer likely led to falsely high  $^3\text{H}$  rates at ETNP conditions (see section 1) and contributed to the systematic difference between our  $^3\text{H}$  and LL  $^{14}\text{C}$  parallel rates. Because the LL 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



**Figure 5.** Profiles of methane, and LL <sup>14</sup>C and <sup>3</sup>H methane oxidation rates down to 2500 m in the water column for Stations 1, 4a, and 8. The error bars for the LL <sup>14</sup>C and <sup>3</sup>H rates are the same as in Figure 4, and the gray boxes show the OMZ depth interval bounded by 10 μmol O<sub>2</sub> L<sup>-1</sup>.

adds ~300 times less CH<sub>4</sub> to samples and uses ~10<sup>6</sup> times less radioactivity than the <sup>3</sup>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<sub>10</sub> correction to the rate data to account for this issue (see section 2). In this study specifically, the large filter pore size (1.2 μm) may have led to falsely low LL <sup>14</sup>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 <sup>14</sup>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.

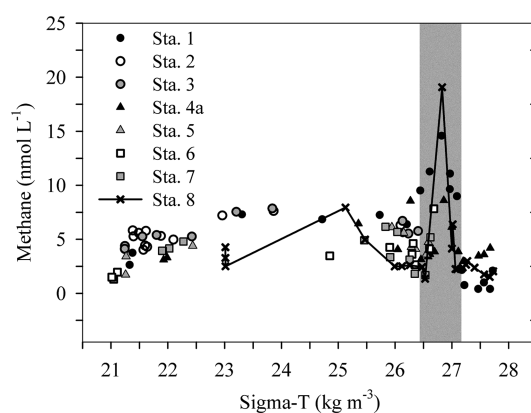
The combination of the parallel LL <sup>14</sup>C and <sup>3</sup>H rates from this study and the previous coastal study show a well-defined trend, albeit with some scatter, of a greater difference between methods at slower MO<sub>x</sub> rates (Figure 6). Disagreement between parallel rate measurements has also been reported for the HL <sup>14</sup>C-CH<sub>4</sub> and <sup>3</sup>H-CH<sub>4</sub> tracers. A study in the Black Sea reported HL <sup>14</sup>C rates that were 0.1–350 (median: 2.1)



**Figure 6.** <sup>3</sup>H oxidation rates plotted against the corresponding parallel LL <sup>14</sup>C rates from this study and the previous coastal study [Pack *et al.*, 2011]. Parallel rates using HL <sup>14</sup>C and <sup>3</sup>H in the Black Sea water column [Reeburgh *et al.*, 1991] are also plotted. Error bars for the LL <sup>14</sup>C and <sup>3</sup>H rates from this study are the same as in Figure 4.

times faster than the parallel <sup>3</sup>H rates (Figure 6, data from Reeburgh *et al.* [1991]). A more recent study in an Arctic fjord observed <sup>3</sup>H rates > HL <sup>14</sup>C rates in ~80 nmol L<sup>-1</sup> CH<sub>4</sub> waters, but HL <sup>14</sup>C rates > <sup>3</sup>H rates in waters with 20 nmol L<sup>-1</sup> CH<sub>4</sub> [Mau *et al.*, 2013]. The study suggested that the 440–540 nmol L<sup>-1</sup> increase in CH<sub>4</sub> from the HL <sup>14</sup>C tracer (as opposed to the 1–2 nmol L<sup>-1</sup> increase from the <sup>3</sup>H tracer) led to priming in the 20 nmol L<sup>-1</sup> CH<sub>4</sub> waters. While in the 80 nmol L<sup>-1</sup> waters, the enzyme machinery associated with MO<sub>x</sub> was apparently at a lower concentration than in the 20 nmol L<sup>-1</sup> waters and became saturated with CH<sub>4</sub> from the HL <sup>14</sup>C tracer addition. This situation yielded <sup>3</sup>H rates > HL <sup>14</sup>C rates (see the discussion in Mau *et al.* [2013]).

When planning future water column MO<sub>x</sub> rate measurements, the <sup>3</sup>H, LL <sup>14</sup>C, and HL <sup>14</sup>C methods are all available tools, and their respective strengths and weaknesses should be considered.



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

The advantage of the  $^3\text{H}$  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  $\text{LL}^{14}\text{C}$  method avoids the use of radiation vans but is costlier and requires more laboratory processing time than the  $^3\text{H}$  method. Taking these strengths and weakness together, the  $^3\text{H}$  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  $\text{LL}^{14}\text{C}$  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).

An additional advantage of the  $\text{LL}^{14}\text{C}$  method is that it can be used to determine the partitioning of methane-carbon between  $\text{CO}_2$  and cell biomass (the  $^3\text{H}$  method cannot accomplish this). Carbon (C) partitioning provides valuable data for C-cycle studies and for establishing C budgets. The  $\text{HL}^{14}\text{C}$  method can also provide C-partitioning data, its sample processing time lays between the  $^3\text{H}$  and  $\text{LL}^{14}\text{C}$  methods (due to filtering and  $\text{CO}_2$  collection steps), it is costwise similar to the  $^3\text{H}$  method, but it requires a radiation van and has the greatest potential for priming samples. Thus, its most practical use is in high- $\text{CH}_4$  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  $\text{CH}_4$  dynamics, we will assume the parallel  $^3\text{H}$  and  $\text{LL}^{14}\text{C}$  rate data bracket the actual range of  $\text{MO}_x$  rates, with the  $\text{LL}^{14}\text{C}$  as the minimum (underestimate due to loss of biomass through large pore size filters) and the  $^3\text{H}$  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  $\text{CH}_4$  budgets.

#### 4.2. ETNP Environmental Controls on Methane and $\text{MO}_x$ Rates

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

Our  $\text{LL}^{14}\text{C}$  and  $^3\text{H}$  rate data show relationships with a number of environmental parameters and provide insight into the controls on  $\text{MO}_x$  rates in ETNP waters.  $\text{LL}^{14}\text{C}$  fractional turnover rates ( $k$ ) show weak, but statistically significant ( $p < 0.05$ ) linear correlations with the product of  $\text{CH}_4$  and  $\text{O}_2$  concentrations ( $\text{CH}_4 \times \text{O}_2$ ),  $\text{O}_2$ , temperature, salinity, and density (Table 3).  $^3\text{H}$  values of  $k$  show statistically significant, although weaker, linear correlations with  $\text{O}_2$ , temperature, salinity, and density (Table 3). The strongest linear correlation appears between the  $\text{LL}^{14}\text{C}$  values of  $k$  and  $\text{CH}_4 \times \text{O}_2$  ( $r^2 = 0.67$ ,  $\text{df} = 43$ , Figure 8 and Table 3). This suggests that the combined availability of  $\text{CH}_4$  and  $\text{O}_2$  (i.e., second-order kinetic control) may influence  $\text{MO}_x$  rates in the ETNP region.

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

Parameter	LL <sup>14</sup> C <i>k</i> (d <sup>-1</sup> )			<sup>3</sup> H <i>k</i> (d <sup>-1</sup> )		
	Slope × 10 <sup>-5</sup>	r <sup>2</sup>	<i>p</i>	Slope × 10 <sup>-5</sup>	(r <sup>2</sup> ) <sup>a</sup>	<i>p</i> <sup>b</sup>
CH <sub>4</sub> × O <sub>2</sub> (nmol L <sup>-1</sup> × μmol L <sup>-1</sup> )	0.13	0.67	<0.0001	0.8	0.11	0.026
CH <sub>4</sub> (nmol L <sup>-1</sup> )	2.0	0.00	0.84	-7.8	0.02	0.37
O <sub>2</sub> (μmol L <sup>-1</sup> )	0.46	0.54	<0.0001	4.3	0.22	0.0011
Temperature (°C)	4.3	0.54	<0.0001	66	0.32	<0.0001
Salinity	-70	0.49	<0.0001	-540	0.25	0.0005
Density (sigma-theta, kg m <sup>-3</sup> )	-17	0.61	<0.0001	-210	0.32	<0.0001

<sup>a</sup>The coefficient of determination for each linear regression.

<sup>b</sup>*P* value to evaluate the statistical significance of each linear regression.

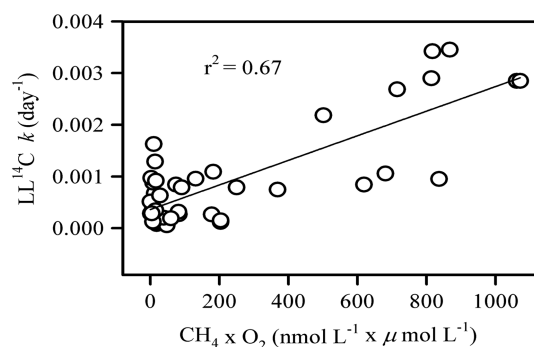
Both CH<sub>4</sub> and O<sub>2</sub> concentrations must be limiting (i.e., close to their respective half-saturation constants) for second-order kinetic control. Methane is likely limiting in all of our samples because it ranges from 0.27 to 19 nmol L<sup>-1</sup> and the CH<sub>4</sub> half-saturation constants reported for marine methanotrophs are ≥60 nmol L<sup>-1</sup> [Ward and Kilpatrick, 1990]. In contrast, O<sub>2</sub> 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 1 μmol L<sup>-1</sup> O<sub>2</sub> 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 <sup>3</sup>H and LL <sup>14</sup>C turnover times (*τ*, the reciprocal of *k*, equation (2)) occur at colder water temperatures, lower CH<sub>4</sub> concentrations, and higher salinities and densities, while faster turnover times are found at warmer temperatures, higher CH<sub>4</sub>, and lower salinities and densities (Figure 9a–9h). This relationship with CH<sub>4</sub> has been reported with previous <sup>3</sup>H rate measurements [Valentine *et al.*, 2001; Mau *et al.*, 2012] and may indicate the existence of less active methanotroph communities at lower CH<sub>4</sub> 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. MO<sub>x</sub> rates were not used to investigate environmental correlations because rate calculations incorporate in situ CH<sub>4</sub> concentrations (equation (1)) and thus mathematically correlate MO<sub>x</sub> rates with CH<sub>4</sub> concentration.

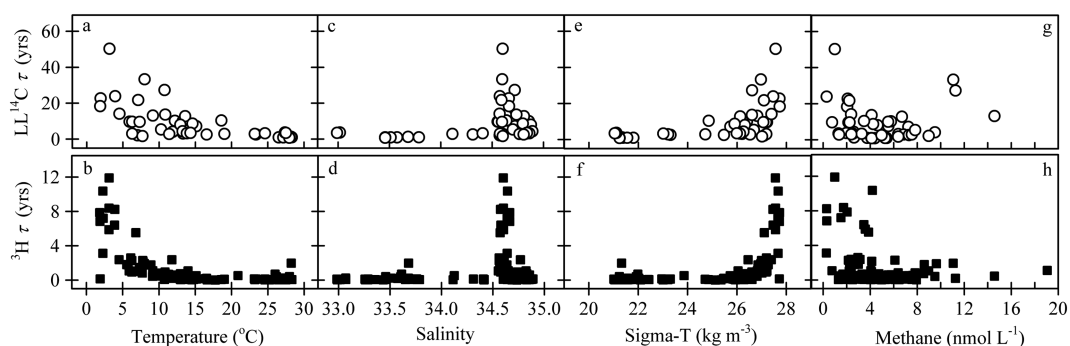
### 4.3. Regional MO<sub>x</sub> Within the ETNP OMZ

Depth-integrated MO<sub>x</sub> rates can provide regional estimates of CH<sub>4</sub> oxidation. In order to examine CH<sub>4</sub> consumption within the OMZ, where the ETNP's large CH<sub>4</sub> pool resides, MO<sub>x</sub> rates were depth integrated from 200 to 760 m and multiplied by the ETNP area (5.2 × 10<sup>6</sup> km<sup>2</sup>) [Sansone *et al.*, 2001]. These calculations yield regional sink estimates of 4.5–6.1 × 10<sup>10</sup> and 26–100 × 10<sup>10</sup> g CH<sub>4</sub> yr<sup>-1</sup> for the LL <sup>14</sup>C and <sup>3</sup>H methods, respectively, and show that

substantial methanotrophic activity in the OMZ consumes CH<sub>4</sub> at depth, thereby preventing its eventual release to the atmosphere. MO<sub>x</sub> in the OMZ over the ETNP area consumes more CH<sub>4</sub> than that reported for the Eel River Basin (2 × 10<sup>6</sup> g CH<sub>4</sub> yr<sup>-1</sup>) [Valentine *et al.*, 2001] and the Juan de Fuca Ridge hydrothermal plume (1.3 × 10<sup>9</sup> g CH<sub>4</sub> yr<sup>-1</sup>) [de Angelis *et al.*, 1993], regions that are actively venting CH<sub>4</sub>, but cover much smaller areas (25 km<sup>2</sup> and 170 km<sup>2</sup>, respectively). These data show that slower MO<sub>x</sub> rates can indeed integrate to a significant CH<sub>4</sub> sink if functioning throughout a large water volume.



**Figure 8.** Plot of LL <sup>14</sup>C fractional turnover rates (*k*) versus the product of methane and oxygen concentrations (CH<sub>4</sub> × O<sub>2</sub>).



**Figure 9.** Plots of LL  $^{14}\text{C}$  and  $^3\text{H}$  turnover times ( $\tau$ ) versus (a, b) temperature, (c, d) salinity, (e, f) density, and (g, h) methane.

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  $0.39\text{--}0.44 \times 10^{11} \text{ g CH}_4 \text{ yr}^{-1}$  (LL  $^{14}\text{C}$ ) or  $0.56\text{--}2.6 \times 10^{11} \text{ g CH}_4 \text{ yr}^{-1}$  ( $^3\text{H}$ ) are consumed in this interval of the OMZ. We expect that the amount of  $\text{CH}_4$  contributed from coastal sediments to this depth interval is similar to the amount of  $\text{CH}_4$  consumed within the interval. This assumes that  $\text{CH}_4$  concentrations are not changing with time and that the rate of  $\text{CH}_4$  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  $\text{CH}_4$  diffusing from coastal sediments ( $0.24$  and  $1.7 \mu\text{mol m}^{-2} \text{ d}^{-1}$ , 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  $\sim 17,800 \text{ km}^2$  area of coastal sediment could potentially supply  $\text{CH}_4$  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  $0.25\text{--}1.8 \times 10^8 \text{ g CH}_4 \text{ yr}^{-1}$ , 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  $\text{CH}_4$  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  $\text{CH}_4$  maximum contributes 25% of the ocean's estimated  $\text{CH}_4$  source to the atmosphere [Reeburgh, 2007]. The maximum is thought to be comprised of  $\text{CH}_4$  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  $\text{CH}_4$  to the atmosphere (e.g., Nihous and Masutani [2006]), but previous studies have suggested that sea-air flux is the main sink for  $\text{CH}_4$  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 ( $\sim 0\text{--}200$  m, through the subsurface maximum) show that  $0.25\text{--}1.3$  and  $17\text{--}47 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ , for the LL  $^{14}\text{C}$  and  $^3\text{H}$  methods, respectively, are consumed in ETNP surface waters (Table 4). Whereas, sea-air fluxes of  $\text{CH}_4$  from surface waters, estimated using the techniques outlined in Holmes *et al.* [2000], ranged from  $-0.64$  to  $9.4 \mu\text{mol CH}_4 \text{ m}^{-2} \text{ d}^{-1}$  (Table 4). The negative flux values at Stations 5 and 6 indicate a  $\text{CH}_4$  flux into surface waters, reflecting efficient  $\text{MO}_x$  that consumes upward fluxes of  $\text{CH}_4$  before they reach the atmosphere, as well as some amount of atmospheric  $\text{CH}_4$ . 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  $\text{CH}_4$  losses in ETNP surface waters and shows that  $\text{MO}_x$  on average makes up 25% (LL  $^{14}\text{C}$ ) or 85% ( $^3\text{H}$ ) of these losses. The results, from both the LL  $^{14}\text{C}$  and  $^3\text{H}$  methods, show that methanotrophy is a significant sink for  $\text{CH}_4$  in ETNP surface waters and that it mitigates the release of  $\text{CH}_4$  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  $\text{CH}_4$  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,



**Table 4.** ETNP Surface Layer Depth-Integrated  $\text{MO}_x$  Rates, Sea-Air Flux Estimates, and Fraction of ETNP  $\text{CH}_4$  Surface Losses Made Up by  $\text{MO}_x^a$ 

Station	Depth Range <sup>b</sup> (m)	Ing. $\text{MO}_x$ Rates		Sea-Air Flux ( $\mu\text{mol CH}_4 \text{ d}^{-1} \text{ m}^2$ )	$\text{MO}_x$ Fraction <sup>d</sup>	
		LL $^{14}\text{C}$	$^3\text{H}$		LL $^{14}\text{C}$	$^3\text{H}$
		( $\mu\text{mol CH}_4 \text{ d}^{-1} \text{ m}^2$ )			%	
1	6–201	1.2	17	2.1	36.2	89.2
2	4–199	na	47	9.4	na	83.4
3	4–201	1.2	24	5.5	17.7	81.5
4a	10–242	na	25	3	na	89.2
5	5–170	na	40	−0.16	<sup>c</sup>	<sup>c</sup>
6	4–190	0.25	19	−0.64	<sup>c</sup>	<sup>c</sup>
7	5–200	na	20	5	na	80.1
8	5–233	1.3	38	5.6	18.5	87.2

<sup>a</sup>The sea-air fluxes were calculated using the methods outlined in *Holmes et al.* [2000] and an average December 2008 wind speed of  $5.8 \text{ m s}^{-1}$  from the National Data Buoy Center (<http://www.ndbc.noaa.gov/>, buoy numbers 43301 and 32315).

<sup>b</sup>The depth range used for integration.

<sup>c</sup>The stations with a negative flux indicate a sea-air  $\text{CH}_4$  flux into the surface waters and efficient  $\text{MO}_x$  that comprises all mixed layer losses.

<sup>d</sup> $\text{MO}_x$  fraction =  $100 \times \text{Ing. MO}_x \text{ rates} / (\text{Ing. MO}_x \text{ rates} + \text{Sea-air flux})$ .

although slow, still provide a significant internal  $\text{CH}_4$  sink that limits the flux of  $\text{CH}_4$  from ocean to atmosphere. Future improvements in rate measurement methods, especially if they confirm the ability to measure very low rates with the LL  $^{14}\text{C}$  tracer, will further improve our understanding of the role of  $\text{MO}_x$  in  $\text{CH}_4$  ocean-atmosphere fluxes and how they may change in the future when OMZ's are expected to expand.

Several improvements to both the  $^3\text{H}$  and LL  $^{14}\text{C}$  methods can be recommended for future  $\text{MO}_x$  rate measurements. The  $^3\text{H}$  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 LL  $^{14}\text{C}$  method, filters with a pore size of  $0.2 \mu\text{m}$  should be used for capturing the bacterial biomass. For both methods, if a  $Q_{10}$  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  $Q_{10}$  correction. These easily implement updates that should improve  $^3\text{H}$  and LL  $^{14}\text{C}$  parallel rate measurements; however, highly controlled parallel  $^3\text{H}$  and LL  $^{14}\text{C}$  rate experiments are required to identify the underlying cause(s) of any remaining discrepancies in parallel rates. Overall, more work is needed to bring all three tracer-based rate measurements ( $^3\text{H}$ , HL  $^{14}\text{C}$ , and LL  $^{14}\text{C}$ ) in line, and accomplishing this is essential for establishing accurate  $\text{CH}_4$  budgets in the ocean water column.

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#### References

- Burke, R. A., D. F. Reid, J. M. Brooks, and D. M. Lavoie (1983), Upper water column methane geochemistry in the eastern tropical North Pacific, *Limnol. Oceanogr.*, *28*, 19–32, doi:10.4319/lo.1983.28.1.0019.
- Ciais, P., et al. (2013), Carbon and other biogeochemical cycles, in *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by T. F. Stocker et al., pp. 465–570, Cambridge Univ. Press, U. K., and New York.
- Cicerone, R. J., and R. S. Oremland (1988), Biogeochemical aspects of atmospheric methane, *Global Biogeochem. Cycles*, *2*, 299–327, doi:10.1029/GB002i004p00299.
- Coleman, D., J. Risatti, and M. Schoel (1981), Fractionation of carbon and hydrogen isotopes by methane oxidizing bacteria, *Geochim. Cosmochim. Acta*, *45*, 1033–1037, doi:10.1016/0016-7037(81)90129-0.
- de Angelis, M. A., M. D. Lilley, E. J. Olson, and J. A. Baross (1993), Methane oxidation in deep sea hydrothermal plumes of the Endeavour Segment of the Juan de Fuca Ridge, *Deep Sea Res., Part I*, *40*, 1169–1186, doi:10.1016/0967-0637(93)90132-m.
- Denman, K. L., et al. (2007), Couplings between changes in the climate system and biogeochemistry, in *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, edited by S. Solomon et al., pp. 500–587, Cambridge Univ. Press, U. K., and New York.
- Devol, A. H. (1978), Bacterial oxygen uptake kinetics as related to biological processes in oxygen deficient zones of the oceans, *Deep Sea Res.*, *25*, 137–146.
- Dickens, G. R. (2003), Rethinking the global carbon cycle with a large, dynamic and microbially mediated gas hydrate capacitor, *Earth Planet. Sci. Lett.*, *213*, 169–183.
- Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov (2001), Effects of size and temperature on metabolic rate, *Science*, *293*, 2248–2251, doi:10.1126/science.1061967.

- Griffiths, R. P., B. A. Caldwell, J. D. Cline, W. A. Broich, and R. J. Morita (1982), Field observations of methane concentrations and oxidation rates in the Southeastern Bering Sea, *Appl. Environ. Microbiol.*, *44*, 435–446.
- Heeschen, K. U., R. S. Keir, G. Rehder, O. Klatt, and E. Suess (2004), Methane dynamics in the Weddell Sea determined via stable isotope ratios and CFC-11, *Global Biogeochem. Cycles*, *18*, GB2012, doi:10.1029/2003GB002151.
- Heintz, M. B. (2011), Rates of aerobic methane oxidation in the waters of the Santa Monica Basin and Alaskan Arctic Lakes measured with a tritium-based radiotracer technique, PhD thesis, Dep. of Earth Sci., Univ. of Calif., Santa Barbara.
- Heintz, M. B., S. Mau, and D. L. Valentine (2012), Physical control on methanotrophic potential in waters of the Santa Monica Basin, Southern California, *Limnol. Oceanogr.*, *57*, 420–432, doi:10.4319/lo.2012.57.2.0420.
- Hinrichs, K.-U., and A. Boetius (2002), The anaerobic oxidation of methane: New insights in microbial ecology and biogeochemistry, in *Ocean Margin Systems*, edited by G. Wefer et al., pp. 457–477, Springer, Berlin, doi:10.1007/978-3-662-05127-6\_28.
- Holmes, M. E., F. J. Sansone, T. M. Rust, and B. N. Popp (2000), Methane production, consumption, and air-sea exchange in the open ocean: An evaluation based on carbon isotopic ratios, *Global Biogeochem. Cycles*, *14*, 1–10, doi:10.1029/1999GB001209.
- Houweling, S., F. Dentener, and J. Lelieveld (2000), Simulation of preindustrial atmospheric methane to constrain the global source of natural wetlands, *J. Geophys. Res.*, *105*, 17,243–17,255, doi:10.1029/2000JD900193.
- Jones, R. D. (1991), Carbon monoxide and methane distribution and consumption in the photic zone of the Sargasso Sea, *Deep Sea Res., Part 1*, *38*, 625–635, doi:10.1016/0198-0149(91)90002-w.
- Karl, D. M. (1999), A sea of change: Biogeochemical variability in the North Pacific subtropical gyre, *Ecosystems*, *2*, 181–214.
- Karl, D. M., and B. D. Tilbrook (1994), Production and transport of methane in oceanic particulate organic matter, *Nature*, *368*, 732–734, doi:10.1038/368732a0.
- Karl, D. M., L. Beversdorf, K. M. Björkman, M. J. Church, A. Martinez, and E. F. DeLong (2008), Aerobic production of methane in the sea, *Nat. Geosci.*, *1*, 473–478, doi:10.1038/ngeo234.
- Keir, R. S., J. Greinert, M. Rhein, G. Petrick, J. Sültenfuß, and K. Fühaupter (2005), Methane and methane carbon isotope ratios in the Northeast Atlantic including the Mid-Atlantic Ridge (50°N), *Deep Sea Res., Part 1*, *52*(6), 1043–1070, doi:10.1016/j.dsr.2004.12.006.
- Lamontagne, R. A., J. W. Swinnerton, V. J. Linnenbom, and W. D. Smith (1973), Methane concentrations in various marine environments, *J. Geophys. Res.*, *78*, 5317–5324, doi:10.1029/JC078i024p05317.
- Mau, S., M. B. Heintz, and D. L. Valentine (2012), Quantification of CH<sub>4</sub> loss and transport in dissolved plumes of the Santa Barbara Channel, California, *Cont. Shelf Res.*, *32*, 110–120, doi:10.1016/j.csr.2011.10.016.
- Mau, S., J. Blees, E. Helmke, H. Niemann, and E. Damm (2013), Vertical distribution of methane oxidation and methanotrophic response to elevated methane concentrations in stratified waters of the Arctic fjord Storfjorden (Svalbard, Norway), *Biogeosciences*, *10*(10), 6267–6278, doi:10.5194/bg-10-6267-2013.
- Mendes, S. D., M. C. Redmond, K. Voiggritter, C. Perez, R. Scarlett, and D. L. Valentine (2015), Marine microbes rapidly adapt to consume ethane, propane, and butane within the dissolved hydrocarbon plume of a natural seep, *J. Geophys. Res. Oceans*, *120*, 1937–1953, doi:10.1002/2014JC0.10362.
- Naqvi, S. W. A., H. W. Bange, L. Farias, P. M. S. Monteiro, M. I. Scranton, and J. Zhang (2010), Marine hypoxia/anoxia as a source of CH<sub>4</sub> and N<sub>2</sub>O, *Biogeosciences*, *7*(7), 2159–2190, doi:10.5194/bg-7-2159-2010.
- Nihous, G. C., and S. M. Masutani (2006), A model of methane concentration profiles in the open ocean, *J. Mar. Res.*, *64*, 629–650, doi:10.1357/002224006778715748.
- Pack, M. A., M. B. Heintz, W. S. Reeburgh, S. E. Trumbore, D. L. Valentine, X. Xu, and E. R. M. Druffel (2011), A method for measuring methane oxidation rates using low-levels of <sup>14</sup>C-labeled methane and accelerator mass spectrometry, *Limnol. Oceanogr. Methods*, *9*, 245–260, doi:10.4319/lom.2011.9.245.
- Pennington, J. T., K. L. Mahoney, V. S. Kuwahara, D. D. Kolber, R. Calienes, and F. P. Chaver (2006), Primary production in the eastern tropical Pacific: A review, *Prog. Oceanogr.*, *69*, 285–317, doi:10.1016/j.pocean.2006.03.012.
- Reeburgh, W. S. (2007), Oceanic methane biogeochemistry, *Chem. Rev.*, *107*, 486–513, doi:10.1021/cr050362v.
- Reeburgh, W. S., B. B. Ward, S. C. Whalen, K. A. Sandbeck, K. A. Kilpatrick, and L. J. Kerkhof (1991), Black-sea methane geochemistry, *Deep Sea Res., Part A*, *38*, S1189–S1210, doi:10.1016/S0198-0149(10)80030-5.
- Rehder, G., R. S. Keir, E. Suess, and M. Rhein (1999), Methane in the northern Atlantic controlled by microbial oxidation and atmospheric history, *Geophys. Res. Lett.*, *26*(5), 587–590, doi:10.1029/1999GL900049.
- Sansone, F. J., B. N. Popp, A. Gasc, A. W. Graham, and T. M. Rust (2001), Highly elevated methane in the eastern tropical north Pacific and associated isotopically enriched fluxes to the atmosphere, *Geophys. Res. Lett.*, *28*, 4567–4570, doi:10.1029/2001GL013460.
- Sansone, F. J., A. W. Graham, and W. M. Berelson (2004), Methane along the western Mexican margin, *Limnol. Oceanogr.*, *49*, 2242–2255, doi:10.4319/lo.2004.49.6.2242.
- Schlitzer, R. (2010), Ocean data view. [Available at <http://odv.awi.de/>].
- Scranton, M. I., and P. G. Brewer (1978), Consumption of dissolved methane in the deep ocean, *Limnol. Oceanogr.*, *23*(6), 1207–1213, doi:10.4319/lo.1978.23.6.1207.
- Valentine, D. L., D. C. Blanton, W. S. Reeburgh, and M. Kastner (2001), Water column methane oxidation adjacent to an area of active hydrate dissociation, Eel River Basin, *Geochim. Cosmochim. Acta*, *65*, 2633–2640, doi:10.1016/S0016-7037(01)00625-1.
- Ward, B. B., and K. A. Kilpatrick (1990), Relationship between substrate concentration and oxidation of ammonium and methane in a stratified water column, *Cont. Shelf Res.*, *10*, 1193–1208, doi:10.1016/0278-4343(90)90016-F.
- Ward, B. B., K. A. Kilpatrick, P. C. Novelli, and M. I. Scranton (1987), Methane oxidation and methane fluxes in the ocean surface-layer and deep anoxic waters, *Nature*, *327*, 226–229, doi:10.1038/327226a0.
- Ward, B. B., K. A. Kilpatrick, A. E. Wopat, E. C. Minnich, and M. E. Lidstrom (1989), Methane oxidation in Saanich Inlet during summer stratification, *Cont. Shelf Res.*, *9*, 65–75, doi:10.1016/0278-4343(89)90083-6.
- Wuebbles, D. J., and K. Hayhoe (2002), Atmospheric methane and global change, *Earth Sci. Rev.*, *57*, 177–210, doi:10.1016/S0012-8252(01)00062-9.
- Xu, X., S. E. Trumbore, S. H. Zheng, J. R. Southon, K. E. McDuffee, M. Luttgen, and J. C. Liu (2007), Modifying a sealed tube zinc reduction method for preparation of AMS graphite targets: Reducing background and attaining high precision, *Nucl. Instrum. Methods Phys. Res., Sect. B*, *259*, 320–329, doi:10.1016/j.nimb.2007.01.175.
- Yamamoto, S., J. B. Alcauskas, and T. E. Crozier (1976), Solubility of methane in distilled water and seawater, *J. Chem. Eng. Data*, *21*, 78–80, doi:10.1021/je60068a029.
- Zhang, X., K. C. Hester, W. Ussler, P. M. Walz, E. T. Peltzer, and P. G. Brewer (2011), In situ Raman-based measurements of high dissolved methane concentrations in hydrate-rich ocean sediments, *Geophys. Res. Lett.*, *38*, L08605, doi:10.1029/2011GL04714.