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Dynamics and controls of methane oxidation in a Danish wetland sediment *

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1. SUMMARY

The patterns and controls of methane oxidation in a Danish wetland sediment have been determined using a combination of slurry and intact core techniques. Results from slurries indicated that methane oxidation was effectively inhibited by low concentrations of nitrapyrin (9 μM) and acetylene (0.5 μM) but that oxidation was relatively insensitive to pH between 6 and 8; in addition, high concentrations of ammonia (1 mM) decreased oxidation, especially at alkaline pH. Kinetic analyses of methane oxidation in slurries indicated that V_{max} was high relative to values reported for other sediments, that V_{max} changed seasonally, that K_m was consistently low (2–4 μM) and that threshold values were low (3–5 nM) but insufficient to allow consumption of atmospheric methane. Analyses based on intact cores indicated that the extent of methane oxidation was highly dependent on oxygen availability, particularly as affected by benthic photosynthesis or

the presence of algal mats. Methane emission and oxidation showed a light saturation response above 400 $\mu\text{Einsteins m}^{-2} \text{s}^{-1}$. Both core and slurry analyses indicated that even short periods of anoxia resulted in losses of the capacity for methane oxidation after re-exposure to air. On the other hand, the presence of anoxia-insensitive organisms provided for a significant residual post-anoxia activity. Results from addition of nitrapyrin to the surface of intact cores indicated that shifts in methane emissions coincident with short-term changes in the availability of oxygen were the result of changes in methane oxidation, not methanogenesis.

2. INTRODUCTION

In contrast to methane production, information on methane oxidation *in situ* is limited, especially with respect to the control of methane fluxes from sediments by methane oxidation. Many of the data on ecological aspects of methane oxidation are derived from studies of the water columns of lakes or marine environments [1–7]. These studies have shown that methane oxidation is an active process that can consume more than 90% of the diffusive methane fluxes from anoxic hypolimnia

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to oxic epilimnia. Results from studies of the water column also indicate that rates of methane oxidation may be determined by dissolved oxygen and nitrogen concentrations, that populations of both nitrifying and methanotrophic bacteria consume methane, and that oxidation may occur under anaerobic conditions. Other more recent studies have been conducted with tropical and temperate forest soils [8–12]. These investigations have demonstrated the capacity of soils to consume atmospheric methane and that rates of methane consumption are affected by soil nitrogen content [10].

Anaerobic methane oxidation in various marine systems has dominated the focus of studies conducted with sediments [e.g. refs. 13–16] in spite of the fact that marine environments are not significant sources of methane on a global or even regional basis [17]. Considerably less is known about methane oxidation in freshwater systems such as wetlands and rice paddies which together account for as much as 50% of the total annual atmospheric methane flux [17–19]. In these systems a large fraction of the methane flux traverses a typically oxic sediment-water or sediment-root interface where aerobic oxidation may have a major impact. Work by Lidstrom and Somers [20] and Kuivilla et al. [21] with sediments from Lake Washington, Frenzel et al. [22] with sediments from Lake Constance, and Remsen et al. [23] with sediments from Lake Superior has documented relationships between methane oxidation and oxygen distribution, some of the kinetic aspects of oxidation and the relative extent of methane fluxes versus consumption. DeBont et al. [24], Holzapfel-Pschorn et al. [25], and Schütz et al. [26] have also observed methane oxidation in rice paddy soils and estimated that this process may consume at least 90% of the potential methane flux.

Although the results from lake and rice paddy studies have provided valuable insights, there remain numerous questions about the nature and controls of methane oxidation in freshwater sediments, particularly in wetlands. Work thus far has been concerned primarily with rates and patterns of emission and the isotopic composition of emitted methane [27–35]. The work reported here establishes some of the kinetic parameters for

methane uptake and some of the responses of methane oxidation to parameters such as pH, ammonia, oxygen and light. In addition, the relative rates of methane oxidation versus methane flux and sources of variability in these rates are documented for a shallow wetland sediment.

3. MATERIALS AND METHODS

3.1. Site description and sampling

Sediments were collected from Vilhemsborg Sø, a small freshwater pond ($z_{\max} = 7$ m) located approximately 15 km south of Aarhøus, Denmark. Samples were obtained in the floodplain of a small stream which entered the pond. The area sampled was typically covered with < 10 cm of water and water levels fluctuated as a function of rainfall and runoff. The site contained patches of aquatic macrophytes, especially *Rorippia amphibia*, and was surrounded at the water's edge by a number of other emergent species. The sediments consisted of a silty sand with a silt layer that was variable but typically about 2 mm thick. During June–August, 1989, an algal mat of about 2 mm thickness dominated by an *Oscillatoria* species covered much of the sediment surface. The sediments contained a gas phase with > 90% methane at depths of about 5 cm; active ebullition was observed throughout the period May–November 1989.

Samples for analyses of methane fluxes and for determinations of dissolved methane depth profiles and potential methane oxidation rates were collected using 3.6 cm inner diameter acrylic core tubes. Cores were returned to the laboratory within 1 h after sampling for processing as described below. Sediments for kinetic analyses and for determination of the response of methane oxidation to pH, ammonia, oxygen, acetylene and 2-chloro-6-trichloromethyl pyridine (nitrapyrin) were obtained from the upper 2 mm of water-saturated sediments immediately adjacent to the water's edge. These samples were collected by carefully scraping the surface with a spatula. After collection, the sediments were distributed to a depth of 1–2 mm in a glass Petri dish for return to the laboratory and processing as described below.

3.2. Flux analyses

Methane fluxes were determined from incubations of sealed core tubes containing 10–15 cm of sediment and a headspace of 50–100 cm³. An unstirred water phase of about 3 mm depth was established above the sediment surface in order to provide a known maximum diffusive boundary layer of a thickness comparable to natural diffusive boundary layers; this approach has been previously compared with stirred systems and found to yield acceptable results for oxygen penetration and flux [35]. Water depths in situ at the specific sites where the cores were obtained were ≤ 2 cm. Methane accumulation in the core headspaces was measured at intervals by obtaining 0.2 ml samples with a needle and syringe via sampling ports in the core stoppers. The methane content of the samples was determined using a Shimadzu 14A equipped with a flame-ionization detector and either a DB-1 or GS-Q widebore capillary column (J&W Scientific). The gas chromatograph was operated at 60 °C with a nitrogen carrier flow rate of 20 ml/min. Cores were incubated at ambient temperature (approximately 25 °C) with other conditions as noted below.

The effects of irradiation on methane flux were determined by using a fiber optic light source with an infrared filter which was introduced through the stoppers of sealed cores that were wrapped with foil to eliminate ambient light [34]. Cores were typically illuminated for varied periods with about 690 $\mu\text{Einsteins m}^{-2} \text{s}^{-1}$ (as determined using a Li-Cor Model 185A Quantum Meter). Incubations were initiated with either a period of illumination followed by a period of darkness or vice versa. The effects of illumination were not dependent on the initial incubation conditions. The effects of varied light intensity were investigated by initiating an incubation in darkness, measuring methane flux for 80–100 min, then increasing light intensity in steps from 90–1090 $\mu\text{Einsteins m}^{-2} \text{s}^{-1}$ with flux measurements at each intensity lasting 80–100 min. A final incubation under dark conditions was used to determine whether or not the initial dark flux rates could be re-established after the light incubations.

Cores incubated in the laboratory as above under aerobic conditions with or without illumina-

tion were also incubated under anaerobic conditions in the dark. Anoxic headspaces were established by flushing the core tubes with nitrogen for approximately 10–15 min after measurements of flux rates under aerobic conditions. A typical experiment involved measurement of methane accumulation during 80–120 min of illumination followed by measurement of accumulation in darkness for an additional 80–120 min. Accumulation was measured for a final 80–120 min period after flushing with nitrogen. In some instances longer incubation periods for both light and dark as well as anoxic conditions were used to establish the stability of the response measured during short-term incubations.

For all flux experiments, rates of methane accumulation were determined from triplicate cores and a regression analysis of methane accumulation as a function of time. In all cases, slopes were tested for significance and for differences among treatments where appropriate. For most rate determinations, at least five time points were available for regression analysis; the product-moment coefficients (r^2) were generally ≥ 0.98 .

3.3. Depth profiles

Triplicate cores collected for determination of the depth distribution of potential methane oxidation were sectioned in the laboratory; weighed subsamples (about 1.0 g fresh weight) of homogenized sediments from the 0–2, 2–5, 5–10, 20–30, and 40–50 mm intervals were placed in 120-ml serum bottles containing 1.0 ml of deionized water as a diluent. After sealing, 0.8 ml of ultrapure methane were injected into the bottles to initiate an analysis of methane oxidation (initial dissolved methane concentrations were approximately 10 μM). The bottle contents were mixed and then the gas phase subsampled by syringe and needle with methane measurements as above. The bottles were incubated at room temperature on a tube roller which produced a thin film of sediment on the tube walls. The results of kinetic analyses (see below) indicated that this method minimized phase transfer limitation [36]. Rates of maximum potential methane oxidation were established by observing the depletion of methane over time and performing a regression analysis for the linear or 'zero'

order' period of uptake. After termination of the experiment, dry weights of the sediment samples were determined by drying for 48 h at 70 °C.

Depth profiles of dissolved methane concentrations in the upper 2 cm of sediment were determined by collecting porewaters from cores returned to the laboratory. Porewaters were collected with a 10- μ l gas-tight syringe (Hamilton, Inc.) the tip of which was positioned accurately using a micro-manipulator. After filling the needle with deionized water, the needle tip was inserted to a desired depth and a volume of porewater, typically 2–3 μ l, was pulled into the syringe barrel. The syringe and needle were quickly removed from the micromanipulator and the entire syringe volume was injected into the gas chromatograph described above. This technique required sampling at discrete intervals which were not vertically continuous as is the case for microelectrode profiling. In order to generate a depth profile, replicate samples were collected with a horizontal separation of several mm. The detector response was calibrated using the same collection method to sample sealed serum bottles containing known partial pressures of methane and a volume of deionized water; methane concentrations in the aqueous phase of the serum bottles were calculated from standard relationships for gas solubility and temperature [37].

3.4. Kinetic analyses

Triplicate samples of homogenized surface sediments (1–2 cm³) were placed in 120-ml serum bottles and diluted with 2 ml of deionized water. After sealing, 0.8 ml of ultrapure methane were injected by syringe and needle. The serum bottles were subsequently incubated at ambient temperature on a tube roller as above. Subsamples of the gas phase were removed at intervals to determine the remaining methane concentration. Sampling continued until at least 90% of the added methane was consumed. The kinetics of methane uptake were analyzed by the linearized form of the integrated Michaelis-Menten equation as recommended by Robinson [38]:

$$\frac{t}{(S_0 - S)} = \frac{K_m}{V_{\max}} * \frac{\ln\left(\frac{S_0}{S}\right)}{S_0 - S} + \frac{1}{v} \quad (1)$$

Alternatively, the progress curve data were fit to a Michaelis-Menten model using a non-linear curve fitting technique available from a software package (Kaleidagraph™, Adelbeck Software) for the MacIntosh (Apple Computer Co.). In addition to estimates of $V_{\max}$ and K_m , the threshold concentrations for methane oxidation were estimated after maximal methane depletion occurred and a stable concentration was observed. The presence of a period during which rates of methane consumption were independent of concentration and a subsequent period during which rates were first-order with respect to methane was interpreted to mean that phase-transfer limitation did not occur during the incubation according to the criteria of Robinson and Tiedje [36].

3.5. Effects of anoxia, pH, ammonia, acetylene and nitrapyrin

The effects of oxygen limitation were examined using homogenized surface sediments that were diluted 1:1 with deionized water; 3 cm³ volumes were dispensed into 120-ml serum bottles which were subsequently sealed. One set of triplicate bottles was left with an aerobic atmosphere while three other sets were flushed with nitrogen and incubated with no added methane for periods of 3, 6, and 12 h. At the end of this period, all bottles were opened and equilibrated with the atmosphere for a period of 1 h; afterwards, the bottles were resealed, amended with 0.8 ml of ultrapure methane, and sampled at intervals for methane concentrations. A companion experiment was conducted using intact cores for which flux rates had been determined under aerobic and then anaerobic conditions as outlined above. After concluding a flux analysis under anoxic conditions, cores were re-aerated and flux rates measured again. The effect of anoxia was expressed as the ratio of the post-anoxia aerobic flux rate to the initial aerobic flux rate.

Homogenized surface sediments were buffered with 10 mM solutions of sodium bicarbonate or carbonate to give final pH values of 6, 7, 8, and 9. The pH of the untreated homogenized sediments was 6.8–7.0. The surface sediments were diluted 1:1 with the buffers and then 4 cm³ volumes of the slurry were dispensed into 120-ml serum bot-

bles which were sealed, amended with methane and sampled at intervals as described for the potential methane oxidation analyses. A parallel set of samples at each of the four pH values was also amended with ammonium chloride to give a final concentration of 1 mM. Each treatment was prepared in duplicate.

The effect of acetylene on methane consumption was determined using homogenized surface sediments prepared by a 1:1 dilution with deionized water; 3-cm³ volumes of the slurry were dispensed into 120-ml serum bottles which were sealed and then amended with 0.8 ml of ultrapure methane and various volumes of acetylene (final dissolved concentrations ranging from approximately 0.5 to 50.0 μM). The bottles were sampled at intervals for methane analysis. Methane consumption was determined in triplicate samples at each acetylene concentration and compared to triplicate controls containing no added acetylene. An additional experiment was conducted similarly with the exception that acetylene was added to the sealed serum bottles after an initial rate of methane consumption had been established. For this experiment the final acetylene concentrations ranged from about 4 μM to 1.2 mM.

The effect of nitrapyrin (Hach Chem. Co., 99% purity) on methane consumption was determined using sediments prepared as for the experiments with acetylene. Prior to addition of methane (0.8 ml), the sediments were amended with nitrapyrin at a final concentration of about 9 μM . The nitrapyrin was prepared from a stock solution containing 10 mg ml⁻¹ in acetone. The stock was diluted in deionized water and then the acetone evaporated in vacuo in order to prepare an aqueous solution for addition to the sediments. Methane concentrations in triplicate serum bottles were analyzed at intervals and compared to triplicate unamended controls.

An additional experiment with nitrapyrin was conducted using intact cores. Triplicate sets of cores were each incubated under dark oxic or dark anoxic conditions. One ml of an aqueous solution of nitrapyrin (about 1 mM) was layered on top of the undisturbed sediments of a third set of triplicate cores incubated under dark oxic conditions. Methane fluxes were determined for each

set as described above. Based on the volume of overlying water, the initial nitrapyrin concentration above the sediment was calculated as about 0.3 mM, a level sufficient to result in the rapid accumulation of concentrations in excess of 5–10 μM over the upper several mm.

4. RESULTS

4.1. Flux analyses

Rates of methane emission varied from 3.4 to 544.3 pmol cm⁻² min⁻¹ during laboratory incubations of cores at ambient temperature. The magnitude of the measured rates depended on the sample source, the presence of algal mats, and the

Table 1

Flux rates under various conditions from cores with or without an algal mat at the sediment surface

Rates are given in $\text{pmol cm}^{-2} \text{ min}^{-1} \pm 1 \text{ S.E. (m)}$

Incubation condition	Mat present	Mat absent
Light, oxic	$84.3 \pm 39.4 (2)$	$49.5 \pm 26.0 (4)$
Dark, oxic	$226.8 \pm 117.4 (3)$	$77.1 \pm 36.8 (6)$
Dark, anoxic	$304.5 \pm 128.2 (3)$	$204.2 \pm 74.1 (6)$

incubation conditions. Methane emissions were substantially lower under illuminated than dark aerobic conditions; highest emission rates were observed under anoxic conditions (Fig. 1a,b). Emission rates from sediments with an intact algal mat at the surface were typically higher than rates from similar sediments without mats (Table 1). In general, emission rates from cores were significantly correlated with the total potential flux, which was measured as emission under anoxic conditions (Fig. 2). Emission under light oxic conditions was related to the total potential flux as follows: light flux = $-1.30 + 0.24 \times \text{total potential flux}$; $r^2 = 0.74$; emission under dark oxic conditions was described by: dark flux = $-41.77 + 0.73 \times \text{total potential flux}$; $r^2 = 0.70$. Emissions were generally stable for periods of 12–24 h dur-

Fig. 2. Light and dark oxic fluxes versus total potential diffusive flux (anoxic flux) for cores incubated as in Fig. 1. Open symbols represent light oxic fluxes, closed symbols represent dark oxic fluxes. For some cores, only dark oxic and total potential fluxes were determined. All data points represent means of triplicate determinations; the figure includes both cores with and without algal mats.

ing incubation with any given set of conditions. Responses to changed conditions (e.g. shifts from light to dark or aerobic to anaerobic) were rapid with stability observed within 20–40 min after a change (Fig. 1a,b).

The extent of illumination at the sediment surface was a major factor affecting the magnitude of methane emission in both laboratory and field incubations. In the laboratory, incubation with $690 \mu\text{Einsteins m}^{-2} \text{ s}^{-1}$ resulted in significantly lower emissions than incubation in the dark (Fig. 1a,b). When algal mats were present, emission increased by a factor of about 2.4–3.7 during darkness. Smaller but significantly enhanced fluxes (1.1–2.3) were also observed during darkness for sediments with no apparent mats (Table 1).

The effect of light was examined further by systematically varying illumination from 0–1090 $\mu\text{Einsteins m}^{-2} \text{ s}^{-1}$. The relationship between methane emission and light intensity was hyperbolic (Fig. 3). Rates of emission, which were estimated from methane accumulation during incubations of 80–100 min at each light intensity, decreased linearly between 0–390 $\mu\text{Einsteins m}^{-2} \text{ s}^{-1}$; at higher light intensities, emissions were constant.

Rates of methane oxidation varied reciprocally with rates of emission (Table 2). Methane oxidation was considered equivalent to the difference

Fig. 3. Methane accumulation as a function of time and light intensity in cores with algal mats at the sediment surface. Data points represent means of triplicate determinations. Arrows indicate light intensities in $\mu\text{Einsteins m}^{-2} \text{ s}^{-1}$. Initial conditions were dark oxic and final were dark anoxic.

between methane emission under anoxic conditions and that under the various light and dark aerobic conditions. This assumption required that no changes occurred in the potential methane flux during the period of aerobic incubation (approximately 9 h). The fact that the initial and final dark aerobic flux rates (476.1 ± 10.9 and 433.9 ± 54.7 $\mu\text{mol cm}^{-2} \text{ min}^{-1}$, respectively) were not statistically different justified this assumption. Rates of methane oxidation increased linearly between 0–390 $\mu\text{Einsteins m}^{-2} \text{ s}^{-1}$ and were constant at higher intensities; thus, 390 $\mu\text{Einsteins m}^{-2} \text{ s}^{-1}$ represented a “saturation” value. Expressed as a percentage, methane oxidation consumed about 16.4% of the maximum potential flux in the dark and 77.4% during illumination with ≥ 390 $\mu\text{Einsteins m}^{-2} \text{ s}^{-1}$.

4.2. Depth profiles

Maximum potential methane-oxidizing activity decreased sharply as a function of sediment depth in cores collected in early September, 1989 (Fig. 4). Rates in the 0–2 mm interval were approximately 8-fold higher than rates for the 5–10 mm interval; no activity was observed below this depth during 48-h incubation. Deeper penetration of methane-oxidizing activity was observed for cores collected in early December, 1989 from a shallow

Fig. 4. Depth profiles of methane oxidation rates (●) measured in September, 1989 and methane concentrations (■) measured in July, 1989 for triplicate cores.

stream-bed (data not shown); the sediments in these cores were primarily a sand as opposed to the sandy silts present in the cores collected earlier. Although potential methane-oxidizing activity penetrated deeper in the sandy cores, the maximum rates were greater in the sandy silts.

While oxygen profiles were not measured during September, profiles measured in situ using oxygen microelectrodes during July, 1989 indicated that penetration depths for the sandy silts were maximally 1–2 mm; sub-surface maxima in oxygen concentrations occurred within the upper 1 mm as a result of benthic photosynthesis (P. Roslev and H. Skovgaard, personal communication). Profiles measured in the laboratory for cores collected during December indicated that penetration depths were < 5 mm in the stream-bed sands. Sub-surface oxygen maxima were also possible, depending on the availability of light (R. Carlton, personal communication).

Depth profiles of methane concentrations measured during July, 1989 showed low values (< 10 μM) which increased relatively slowly over the 0–4 mm depth interval (Fig. 4). Below this depth, concentrations increased more rapidly reaching

Table 2

Summary of methane flux and oxidation rates from triplicate cores with algal mats incubated at varied light intensities as indicated in Fig. 3

Methane flux rates are estimated from the slopes of linear regressions of the methane increases over approximately 80–100 min incubation for each light intensity. Methane oxidation rates are calculated by subtracting the various oxic fluxes from the total potential flux determined under anoxic conditions. Rates are given in $\mu\text{mol cm}^{-2} \text{ min}^{-1} \pm \text{S.E.}$ for emissions. The units for light intensities are $\mu\text{Einsteins m}^{-2} \text{ s}^{-1}$.

Light Intensity	Methane flux	Calculated oxidation
0	476.1 ± 10.9	68.2
90	355.1 ± 24.5	189.2
190	191.1 ± 8.2	353.2
390	129.0 ± 12.5	415.3
690	123.1 ± 4.9	421.2
1090	110.1 ± 17.3	434.2
0, anoxic	544.3 ± 10.6	

values of about 500 μM at 15 mm. The presence of gas bubbles which were primarily methane at 30–50 mm indicated that concentrations increased to levels near saturation (about 1.4 nM at 20 °C). Using estimates of water content (90%) and literature values for diffusion coefficients of methane [35], the diffusive flux from 4 mm to the sediment-water interface was about 26 $\text{pmol cm}^{-2} \text{min}^{-1}$; the calculated flux to the 4 mm depth interval from deeper sediments was 390 $\text{pmol cm}^{-2} \text{min}^{-1}$. Flux rates measured for a similar set of cores assayed 4 days earlier were about 123 and 544 $\text{pmol cm}^{-2} \text{min}^{-1}$ for oxic and anoxic conditions respectively. Differences between the calculated diffusive fluxes and the measured fluxes were likely due to a combination of heterogeneity at the sampling site plus the activity of tubificid worms which were present at the sediment surface and which probably increased fluxes due to advection.

4.3. Kinetic analyses

The uptake of methane added to slurries of surface sediments (0–2 mm) showed both zero- and first-order kinetics in accordance with a simple Michaelis-Menten model. Methane uptake was characterized by relatively high values for V_{max} and low values for K_m (Table 3). The measured V_{max} increased systematically over the sampling interval from April to July while the K_m showed a decreasing tendency. V_{max} values obtained during September, 1989 were lower than values from summer even though all samples were incubated at 23–25 °C. The September results were not obtained by progress curve analysis however, but by determining the rate of methane depletion at

saturation methane concentrations only (e.g. 10 μM).

Threshold values for methane oxidation were estimated from the final, stable methane concentrations remaining in the incubation bottles after > 5 days incubation (Table 3). The addition of methane after uptake had ceased was followed immediately by renewed consumption which indicated that the final concentrations were a reflection of transport limitations and not metabolically inactive bacteria (data not shown). The observed threshold values varied between 2.9–4.7 nM (final dissolved concentrations) or 2.1–3.4 ppm (final gas phase concentrations).

4.4. Effects of anoxia, pH, ammonia and inhibitors

The effects of anoxia on methane oxidation were investigated using both slurries and intact cores. Data from slurries indicated that the absence of oxygen for even relatively short periods resulted in a loss of about 85% of the initial capacity for methane oxidation (Fig. 5). There was, however, an anoxia-tolerant methane-oxidizing activity which accounted for about 15% of the initial capacity and which was unaffected by periods of anoxia from 3–12 h. This lower level of anoxia-tolerant activity was expressed immediately after addition of methane and oxygen as indicated by the absence of a lag in methane consumption.

Fig. 5. Rates of methane oxidation in triplicate slurries from the 0–2 mm interval. Slurries were exposed to anoxic conditions for the periods indicated. After reintroduction of air for 1 h, methane was added to establish post-anoxia oxidation rates.

Table 3

Kinetic parameters for methane oxidation in surface sediments from Vilhelmsborg Se

V_{max} is given in $\mu\text{mol cm}^{-3} \text{day}^{-1}$. All values are means of triplicate determinations.

Date	K_m (μM)	V_{max}	Thrsh (nM)
25 April	2.2	15.9	2.9
1 May	2.8	20.6	
21 July	3.7	34.6	4.7
1 September		17.5	

Table 4

Recovery of methane oxidation from anoxia

Intact cores were incubated under oxic conditions to establish initial rates of methane flux; the cores were then incubated under anoxic conditions to determine a total potential flux from which the initial rate of oxidation was estimated. Oxic conditions were re-established; post-anoxia oxidation rates were calculated from the difference between the previous anoxic and the post-anoxic fluxes. Methane accumulation during each of the oxic and anoxic periods was linear over the time intervals indicated.

Treatment	Percent recovery
Mat-covered sediments 11 h anoxic; 4 h oxic, dark	32.8
Mat covered sediments 12 h anoxic; 11 h oxic, illuminated	66.0
Sandy, "exposed" sediments 10.5 h anoxic; 4 h oxic, dark	82.2

Data from core incubations were generally consistent with results from the slurries (Table 4). Methane oxidation in intact cores from a submerged site decreased by 34.0–67.2% from the initial rates after a period of up to 12 h of anoxia.

Fig. 6. Rate constants for methane oxidation by slurries from the 0–2 mm interval; slurries were incubated with methane to establish uptake rate constants after adjusting pH to the values indicated or after addition of 1 mM ammonium chloride (final concentration). Values shown are means of duplicate determinations; error bars are the standard errors of rate constants determined by regression analysis of the natural logarithm of methane concentrations versus time. Closed bars represent controls and stipled bars ammonia addition.

After the re-establishment of oxic conditions, methane oxidation occurred rapidly and was stable for up to 11 h of aerobic incubation as indicated by linear accumulations of methane in the core headspaces. Cores from a sandy site with the sediment surface exposed to the atmosphere behaved similarly but methane oxidation decreased by only 17.2% after 10.5 h of anoxia.

Shifts in pH had a minimal effect on rate constants for methane oxidation in slurries (Fig. 6). A broad maximum was observed between pH 6–8 with a significant decrease at a pH of 9 for control sediments. In contrast, rate constants decreased with increasing pH in sediments to which 1 mM ammonium chloride had been added (Fig. 6); at pH 7–9, rate constants in the presence of ammonium were 1.6–2 times lower than the controls. Rate constants, rather than rates, were estimated for this comparison since the addition of methane to the slurries was at sub-saturating levels (e.g. about 2 μ M).

Additions of both acetylene and nitrapyrin inhibited methane oxidation. Acetylene caused a complete loss of activity at all concentrations used, including 0.5 μ M (final dissolved concentration).

Fig. 7. Effect of added acetylene on methane oxidation in slurries from the 0–2 mm interval. Methane depletion was followed in slurries to establish an initial uptake rate prior to addition of acetylene (as indicated by the arrow). Acetylene was subsequently added to the gas phase to yield final dissolved concentrations of 4 (○), 43 (▲), 128 (■), 423 (◇), and 1240 (◆) μ M; uptake was compared to an untreated control (●). All treatments were run with triplicates.

Inhibition by acetylene was effective immediately upon addition regardless of whether methane and acetylene were added to slurries simultaneously or sequentially (Fig. 7). Nitrapyrin also effectively inhibited methane oxidation in slurries at a concentration of 9 μM . Addition of nitrapyrin to intact cores effectively inhibited methane oxidation as indicated by increased flux rates. Further, fluxes from cores treated with nitrapyrin were comparable to fluxes from cores incubated anaerobically (184.7 vs. 186.4 $\mu\text{mol cm}^{-2} \text{ min}^{-1}$). These rates were 2-fold higher than cores incubated under dark oxic conditions. Finally, neither methane oxidation nor production were observed for slurries of sediments from 0–2 mm when incubated under anoxic conditions (data not shown).

5. DISCUSSION

Analyses of diffusive methane fluxes with intact cores have proven useful for determining both rates and controls of methane oxidation. Previous studies have usually used either slurry [20] or budgetary approaches [21]. In contrast, Frenzel et al. [22] and Sweerts et al. [39] have measured rates of diffusive methane flux from cores with an aerobic water column which was allowed to become anoxic. In their study, rates of methane oxidation have been calculated from the large and rapid change in flux which occurred at low water column oxygen concentrations. A similar approach has been used in the work reported here with the exception that flux rates have been determined before and after replacing the headspaces of cores with nitrogen. Recently, this approach has also been used successfully to characterize methane oxidation in sediments of the Florida Everglades [35].

Interpretation of the core flux data is dependent in part on the extent to which changes in flux can be attributed to methanogenesis as opposed to methane oxidation. In this study, there is no evidence that methanogenesis was active in the upper, oxygenated layers of sediment. The rapid and linear response of methane fluxes to changes in incubation conditions (e.g. transitions from light

to dark, aerobic to anaerobic) is consistent with minimal changes in methane production. Assuming that methanogenesis was restricted to anoxic sediments, it is possible to calculate the time required for a change in methane production at the bottom of the oxygenated interval to significantly affect diffusive surface fluxes as: $t = x^2 / (\phi D_s)$, where x = the depth to the bottom of the oxic zone and ϕD_s = diffusivity of methane in sediment. Using $\phi D_s = 1.6 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ and $x = 2 \text{ mm}$ (based on depths of oxygen penetration and assays for methanogenesis in surface sediments), $t = 41.7 \text{ min}$. This minimum time is greater than the response times which were typically observed. Furthermore, a stimulation in methane production would yield non-linear methane fluxes until a new and stable concentration gradient was established. Such non-linearities have not been observed over either short or long incubation periods (Fig. 1a,b).

Another indication that changes in methane oxidation are of minor importance for the observed changes in flux is the response to nitrapyrin. Fluxes were equal for cores incubated oxically with nitrapyrin or anoxically without; fluxes for both of these treatments were greater than oxic controls. Since nitrapyrin is a well-known inhibitor of methane oxidation (e.g. ref. 40) and a potential inhibitor of methane production [41], the comparable changes in methane flux for oxic nitrapyrin and anoxic treatments indicate that short-term anoxic incubations eliminate methane oxidation without significantly stimulating methane production. Were methane production stimulated, an increase in flux relative to the nitrapyrin treatment would be anticipated.

Results from the core analyses show that diffusive fluxes of methane at the study site are comparable to flux estimates reported for other shallow, freshwater systems (e.g. refs. 28, 33). More importantly though, the results indicate that methane oxidation consumes a significant but variable fraction of the total potential methane flux. The least amount of oxidation is found during dark incubations of sediments covered by an algal mat while the greatest oxidation occurs when sediments without mats are illuminated (Table 1). Thus, light conditions and small-scale heterogeneity at the sediment surface have pronounced ef-

fects on methane fluxes to the water column or atmosphere. The variability in surface fluxes is due in part to the magnitude of methane consumption and not just downcore differences in methanogenesis. For example, total potential methane fluxes differed by a factor of 1.5 between sediments with and without a mat; in contrast fluxes under oxic light conditions differed by a factor of 1.7 while fluxes under dark oxic conditions differed by a factor of 2.9 (Table 1).

Correlations between light and dark oxic flux, and anoxic (e.g. total potential) fluxes further illustrate the impact of methane oxidation (Fig. 2). For illuminated sediments, the observed surface fluxes are significantly correlated with the total potential fluxes and the relative extent of methane oxidation is constant. This relationship is best explained by the well-documented effect of illumination on benthic photosynthesis and oxygen availability (e.g. refs. 35, 42, 43). In the sediments used for this study, as with many other sediments, both oxygen concentrations and penetration depths are increased by benthic photosynthesis under photic conditions [34]. Apparently, benthic oxygen production is adequate to support a relatively constant level of methane oxidation even when the total potential flux of methane to the oxic zone increases. In other words, when sediments are illuminated, the rate of methane oxidation is not limited by oxygen but rather by methane; this limitation would appear to be approximately first order.

Dark oxic fluxes are also significantly correlated with total potential fluxes but the relative extent of methane oxidation is variable (Fig. 2). A regression analysis indicates that methane oxidation can completely eliminate dark methane fluxes at low total potential flux rates. However, dark fluxes increase linearly with total potential fluxes until methane oxidation becomes a minor (< 30%) component of the potential flux. This relationship is best explained by a varying limitation of oxygen in the absence of benthic photosynthesis. The limitation of oxygen in this case is positively correlated with increasing total potential methane fluxes.

The availability of organic matter could play an important role in determining the relationship be-

tween dark oxic and total potential methane fluxes. For example, relative to bare sediments, increased organic matter associated with algal mats could stimulate methanogenesis while simultaneously increasing total oxygen uptake and decreasing oxygen penetration; a greater demand for oxygen consumption by heterotrophs could limit the availability of oxygen for methanotrophic bacteria thereby enhancing methane fluxes. It is notable that the lowest relative rates of methane oxidation were associated with sediments covered by algal mats while the highest rates were observed for sediments with no mats (Table 1).

Although there is a relatively constant relationship between methane consumption and total potential flux in illuminated sediments, the effect of light is variable as a function of intensity (Fig. 3, Table 2). At low light intensities (< 400 $\mu\text{Einsteins m}^{-2} \text{s}^{-1}$), rates of oxidation increase linearly with increasing intensities. A saturation response is observed above 400 $\mu\text{Einsteins m}^{-2} \text{s}^{-1}$ however, and rates of oxidation become constant. This saturation response is analogous to the photosynthetic response of benthic algae and is even characterized by a saturation intensity comparable to that for algal mats (e.g. refs. 43, 44). Thus, diffusive fluxes of methane across the sediment surface can be expected to vary spatially and temporally with sediment oxygen demand, organic content, benthic photosynthesis and illumination as key variables affecting methane oxidation.

Other important variables affecting rates of methane oxidation are the kinetic characteristics of the methanotrophic organisms themselves (Table 3). In particular, V_{max} , K_m and threshold values for methane uptake define the concentration range to which methanotrophic populations are sensitive and the velocities at which methane can be consumed. In this study, threshold values indicate that the methanotrophic populations are incapable of consuming methane from the atmosphere, even though uptake is possible at levels approaching dissolved concentrations equivalent to an equilibrium with the atmosphere. The inability of methanotrophs in these sediments to consume atmospheric methane is consistent with model predictions of Conrad [45]. Thus, freshwater methanotrophs apparently affect concentra-

tions of atmospheric methane by limiting fluxes rather than by direct consumption. In this regard, freshwater methanotrophs may differ significantly from terrestrial and marine methane-oxidizing bacteria (MOB) which seem to have the capability to consume methane at near to sub-atmospheric concentrations [11,13,46,47]. Whether methane oxidation in soils and the marine water column is due to fundamentally different populations or is regulated by environmental parameters (e.g. low methane concentrations in soils) is not known. It is especially important to resolve this issue however since increases in atmospheric methane concentrations could ultimately be limited by a biological sink that is not currently considered significant.

The K_m values for methane uptake (Table 3) are similar to reports by others for sediments and soils as well as for whole cells. Lidstrom and Somers [20], Kuivilla et al. [21], and Remsen et al. [23] have reported values of 5.1–10.7 μM for several freshwater sediments. Values for a variety of soils and peats range from 2.5–66.2 μM [9,12,47–48] while Joergensen [49] and Joergensen and Degn [50] have reported a range of 0.8–2.0 μM for pure cultures. Overall, this range is consistent with the low methane concentrations that are observed in oxic sediments (e.g. ref. 21, Fig. 4). When K_m data and methane concentrations for specific sites are compared (e.g. this study, Kuivilla et al. [21], Remsen et al. [23]) it is apparent that methane uptake may be pseudo-first-order with respect to methane over much of the oxic zone, even near the oxic-anoxic interface. Thus rates of uptake should be directly proportional to changes in methane concentrations. The relationship between methane emission and total potential flux found in this study (see above and Fig. 2) is consistent with an approximately first order process.

The $V_{\max}$ estimates provide a potentially useful comparative index of the capacity for sediments to oxidize methane. For example, the increase in $V_{\max}$ from April to July followed by a decrease in September (Table 3) may reflect seasonal changes in the biomass or specific activity of MOB. A comparison of $V_{\max}$ values indicates that either MOB biomass or specific activity are about 20-fold

lower in sediments from Lake Washington [21] versus the sediments used in this study. This disparity is consistent with the 10-fold lower methane flux rates reported for Lake Washington [21]. Similarly, $V_{\max}$ and potential methane fluxes are approximately proportionately lower in peats from the Florida Everglades [35]. Thus, $V_{\max}$ may reflect the relative magnitudes of methane fluxes in a variety of sediments.

In addition to kinetic parameters, several environmental variables may determine rates of methane oxidation. For example, oxygen is critical for all MOB described to date [4]. Further, there is no evidence supporting anoxic methane oxidation in freshwater sediments [12]. Thus both the distribution and concentrations of oxygen are primary limiting factors in non-marine systems. While it is clearly necessary to understand the kinetics of oxygen uptake by MOB in sediments, it is also important to understand the response of MOB to changes in oxygen concentrations. This is especially true in sediments where shifts from oxic to anoxic conditions occur on relatively short time scales. Both intact core and slurry data from this study suggest that methane oxidation is significantly affected by even brief (3 h) periods of anoxia (Fig. 5, Table 4). Exposure to anoxia results in losses of a substantial fraction of the capacity for methane oxidation as determined by rates of methane uptake after re-establishing aerobic conditions for up to 12 h. However, the results also indicate the presence of an anoxia-tolerant population which may occur in deeper transition zones, where oxygen concentrations fluctuate in response to changes in benthic photosynthesis or other factors affecting oxygen distribution.

The existence of an anoxia-tolerant population of MOB is also supported by depth profiles of maximum potential methane oxidation rates (Fig. 4). Significant rates of oxidation were observed without an apparent lag at depths (3.5–7.5 mm) well below the maximum for oxygen penetration; this was even more pronounced when using sandy sediments from a stream bed. A similar result has been observed for peats from the Florida Everglades [35]. Maximum oxygen penetration depths of 8 mm have been measured while relatively high

rates of potential methane oxidation were detected at depths > 4 cm. The organisms responsible for the observed activity and the mechanisms by which anoxia is tolerated are not presently clear. Further resolution of the effects of anoxia should provide greater insights into the physiological constraints of MOB in situ and possibly provide evidence for the small-scale localization of populations with differing sensitivities to anoxia.

Variables such as pH and ammonia appear less important than oxygen. Over the commonly encountered pH range of 6–9, the alkaline extreme appears most important as a limiting factor. Decreased activity at a pH ≥ 9 may be the result of a number of biochemical and physiological phenomena, including among others a sensitivity to ammonia. Ammonia, as distinct from ammonium, is an inhibitor of methane monooxygenase [40]. Thus, shifts from circumneutral to alkaline pH values can markedly increase the possibility for ammonia inhibition. This may explain the pH effect in the control sediments used in this study since sediments from the study site contain hundreds of micromolar concentrations of total dissolved ammonia (King, unpublished data). The effect of pH on the ionization of ammonia and the subsequent inhibition of methane monooxygenase is the most likely explanation for the results from the ammonium-amended sediments (Fig. 6).

The effect of ammonia on methane oxidation raises a final issue. The MOB are similar in many respects to the ammonia-oxidizing bacteria [40]. In fact, each group can oxidize the primary substrate of the other; it has even been suggested that MOB are important nitrifiers while AOB are significant methane oxidizers [40]. Although MOB and AOB are sensitive to virtually the same inhibitors, an apparent difference in the degree of sensitivity to acetylene may provide a means for distinguishing between methane oxidation by the two groups. As expected from previous reports, nitrapyrin effectively inhibits methane oxidation, either at low concentrations in slurries (9 μM) or at higher concentrations when added at the surface of intact cores. In contrast, methane oxidation in sediments appears more sensitive to acetylene than pure culture studies have indicated, since complete inhibition occurs at dissolved concentrations as low as

0.5 μM . A similar sensitivity to acetylene has been noted parenthetically for sediments from Lake 227 [2]. These results may indicate that the dominant populations of MOB in situ are more susceptible to acetylene than organisms in culture. As a result, it is unlikely that the effort to distinguish between the activities of AOB and MOB can depend on either nitrapyrin or acetylene; other inhibitors, such as picolinic acid, 2,4-dichloro-6-phenyl phenoxyethyl-diethylamine, and β -diethylamino ethyl-diphenylpropyl acetate [40] have yet to be fully evaluated with sediments.

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