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FEMS Microbiology Ecology 17 (1995) 213–220

FEMS MICROBIOLOGY ECOLOGY

A comparison of two nitrification inhibitors used to measure nitrification rates in estuarine sediments

J.M Caffrey *, L.G. Miller

U.S. Geological Survey 345 Middlefield Rd., MS 496 Menlo Park, CA 94025, USA

Received 12 December 1994; revised 8 May 1995; accepted 8 May 1995

Abstract

Nitrification rates were measured using intact sediment cores from South San Francisco Bay and two different nitrification inhibitors: acetylene and methyl fluoride. Sediment oxygen consumption and ammonium and nitrate fluxes were also measured in these cores. Four experiments were conducted in the spring, and one in the fall of 1993. There was no significant difference in nitrification rates measured using the two inhibitors, which suggests that methyl fluoride can be used as an effective inhibitor of nitrification. Nitrification was positively correlated with sediment oxygen consumption and numbers of macrofauna. This suggests that bioturbation by macrofauna is an important control of nitrification rates. Irrigation by the tube-dwelling polychaete, *Asychis elongata*, which dominates the benthic biomass at this location, appears particularly important. Ammonium fluxes out of the sediment were greatest about one week after the spring bloom, while nitrification peaked about one month later.

Keywords: Nitrification; Sediment; Inhibitor; Estuary

1. Introduction

Microbial nitrification is an important regenerative process in the nitrogen cycle of lakes, estuaries, and marine environments [1–3]. Nitrification in aquatic sediments is often the crucial link between mineralization of organic N and loss of N_2 via denitrification. In sediments where nitrification and denitrification are coupled, nitrification may supply more NO_3^- for denitrification than diffusion from the overlying water [4,5]. In estuarine and near-shore marine sediments, where bioturbation and irrigation by benthic infauna also supply overlying water to the zone of active denitrification, the role of nitrification in supplying NO_3^- is less well understood [6–9]. It is essential to determine nitrification rates in these complex aquatic environments with experimental designs that reflect in situ conditions that potentially affect reaction and transport processes [10].

Sediment nitrification has been estimated using ^{15}N enriched NH_4^+ [1,11], and by measuring NH_4^+ and $NO_3^- + NO_2^-$ profiles and fluxes to the overlying water [7,12]. Nitrification has also been determined by comparing NH_4^+ flux before and after inhibition of the NH_4^+ monooxygenase (AMO) enzyme using acetylene [13,14] or N-serve [15–17]. Such inhibitor studies assume the inhibitor is specific for the AMO enzyme and that the inhibitor is rapidly and com-

* Corresponding author. Tel: +1 (415) 354-3343; Fax: +1 (415) 354-3363; E-mail: jcaffrey@rcamnl.wr.usgs.gov

SSDI 0168-6496(95)00026-7

Table 1
Sampling dates (1993) of nitrification experiments, incubation temperature, and experimental treatments

Date	Temperature (°C)	Treatment	Replicates
April 1	16	control	1
		C_2H_2, 1%	3
April 6	16	control	2
		C_2H_2, 1%	3
		CH_3F, 1%	3
April 22	17	control	1
		C_2H_2, 1%	2
		CH_3F, 1%	2
May 19	17	control	1
		C_2H_2, 1%	3
		CH_3F, 1%	3
		CH_3F, 5%	3
October 19	18	control	3
		C_2H_2, 1%	3
		CH_3F, 1%	3

pletely distributed. N-serve is insoluble in water and must be added with alcohol or acetone, resulting in addition of unwanted carbon [18]. Acetylene (C_2H_2) is highly soluble and acts quickly, but inhibits a number of other microbial processes, including denitrification [18], such that the specificity of C_2H_2 must be determined for each system under investigation [19]. Recent reports of the use of methyl fluoride (CH_3F) as an inhibitor of AMO activity in culture and in soils suggest that this gaseous, highly soluble (1.7 ml/ml) compound may prove useful in sediment nitrification studies [20,21], and may be particularly valuable in inhibiting nitrification without affecting denitrification [21]. In this study, we investigated the suitability of methyl fluoride as a nitrification inhibitor in flux studies of whole cores of estuarine sediments.

2. Materials and methods

A series of nitrification experiments were conducted in April, May and October 1993. Either two or three cores (12.1 cm diameter) were used for each treatment with the nitrification inhibitor (Table 1). Cores were collected at a channel site in South San Francisco Bay (37°34.8′N, 121°14.7′W) using a dart coring system on April 1 and 6 during a spring phytoplankton bloom, and on April 22, May 19 and October 19, 1993. The corer was gently lowered into the sediments to reduce the loss of the surface flocculent layer. Only cores with an undisturbed sediment-water interface and clear overlying water were used for the experiments. The height of water overlying the sediment ranged from 25–35 cm and sediment thickness ranged from 15–20 cm. The cores were held overnight at in situ temperatures, and were gently bubbled with air to maintain oxygen concentrations near saturation (oxygen concentrations in San Francisco Bay rarely fall below 80% of saturation [22]). Before beginning the flux measurements, overlying water in the cores was replaced with fresh bottom water.

Cores were sealed without a headspace with tops equipped with a magnetic stirring system and sampling ports. They were incubated in the dark at ambient temperatures. The first water sample was taken after a 1-h incubation period. Water removed from the cores was replaced with bottom water from the sampling site; this resulted in less than 1% dilution. Water samples were filtered through GF/F filters and frozen for nutrient analysis, or stored unfiltered in glass bottles (7 ml) with Winkler reagents for O_2 analysis by automated potentiometric titration [23]. Nutrient analyses ($NO_3^- + NO_2^-$ and

Table 2
Temperature, salinity, bottom water NH_4^+, NO_3^- and chlorophyll *a* concentrations, and sediment pigment concentrations

Date	Temperature (°C)	Salinity (PSU)	NH_4^+ (μM)	NO_3^- (μM)	Chlorophyll *a* [a] ($\mu g\,l^{-1}$)	Chlorophyll *a* [b] ($\mu g\,cm^{-1}$)	Phaeopigment [b] ($\mu g\,cm^{-2}$)
April 1	15.4	13.8	0.70	0.61	30.6	5.48	18.29
April 6	15.7	18.5	0.65	0.71	24.7	3.98	23.13
April 22	16.1	20.1	0.82	1.31	8.5	0.56	5.32
May 19	17.4	24.5	7.00	20.52	1.0	0.25	5.73
October 19	18.6	29.2	7.12	41.96	1.4	0.32	3.35

[a] Bottom water chlorophyll *a* sampled between 1–2 m above sediment-water interface.
[b] Sediment chlorophyll and phaeopigments from 0–1 cm depth interval.

NH_4^+) were conducted within 2 months of collection on a Technicon II autoanalyzer. $NO_3^- + NO_2^-$ (hereafter referred to as NO_3^-) was analyzed by the automated Cd reduction method and NH_4^+ by a modification of the salicylate-hypochlorite method [24].

Water samples were removed from each core approximately hourly during an 8 h incubation. Bottom water was saturated with acetylene or methyl fluoride at room temperature (approx. 20°C) and then added to cores 4 h after the initial water sample was removed. Between 1 and 2% of the overlying water volume of the core was replaced with inhibitor-saturated bottom water. During the May 19 experiment, there was an additional treatment of 5% CH_3F-saturated water. Acetylene, which was produced from calcium carbide, was bubbled through bottom water for approximately 30 min. During the experiments on April 7 and 23, dissolved C_2H_2 and CH_3F were determined by headspace equilibration and FID gas chromatography [25], and were found to be constant over the 4-h incubations. The nitrification rate was determined by the difference between NH_4^+ flux before and after inhibitor addition. At the end of the experiment, a subcore was taken from each core for determination of chlorophyll *a* and phaeopigment in the top 0.5 cm. The remaining sediment was sieved through 0.5 cm mesh screen to collect macrofauna, that were preserved in 10% buffered formalin, identified and enumerated. SYSTAT [26] was used for an analysis of variance to determine if treatment differences were significant. In addition, correlation analyses ($n = 28$) were used to examine the relationships among benthic NH_4^+ fluxes, nitrification rates, sediment pigment concentrations and macrofauna abundances.

3. Results

On April 1, temperature, salinity and bottom water NH_4^+ and NO_3^- concentrations were minimal, while bottom water chlorophyll *a* concentrations were maximal (Table 2). The peak of the annual spring phytoplankton bloom in San Francisco Bay occurred during this period (the end of March and beginning of April) in 1993 [22]. Temperature, salinity and bottom water NH_4^+ and NO_3^- concentrations steadily increased during the spring sampling period, while bottom water chlorophyll *a* concentrations declined (Table 2), reflecting the gradual recession of the bloom. NH_4^+ and NO_3^- concentrations were highest in October. Sediment pigment concentrations also declined over the spring sampling period and

Table 3
Sediment O_2 consumption (SOC), NH_4^+ and NO_3^- fluxes and macrofaunal biomass in San Francisco Bay sediments

Date	Treatment	SOC (mmol O_2 m^{-2} d^{-1})		NH_4^+ Flux (mmol N m^{-2} d^{-1})		NO_3^- Flux (mmol N m^{-2} d^{-1})		Macrofaunal biomass (g ash free C m^{-2})	
Apr 1	Control	72.5		2.4		−0.1		16.7	
	C_2H_2	30.6	(8.0)	0.8	(0.1)	0.1	(0.1)	14.2	(4.6)
Apr 6	Control	40.2	(8.3)	1.4	(0.3)	6.2	(3.5)	5.6	(1.5)
	C_2H_2	34.8	(3.7)	2.0	(0.6)	0.9	(0.6)	12.1	(3.0)
	CH_3F	25.5	(4.6)	0.5	(0.4)	0.2	(0.2)	4.5	(2.2)
Apr 22	Control	29.5		0.4		−0.2		15.9	
	C_2H_2	47.5	(3.2)	0.6	(0.1)	0.5	(0.2)	10.6	(0.8)
	CH_3F	43.0	(15.3)	0.9	(0.4)	−2.0	(0.9)	5.3	(0.2)
May 19	Control	116.0		5.2		−7.2		32.5	
	C_2H_2	52.5	(7.5)	0.5	(0.9)	1.2	(1.6)	14.4	(1.7)
	CH_3F 1%	71.3	(7.2)	0.5	(0.2)	0.1	(0.5)	24.2	(10.7)
	CH_3F 5%	46.9	(6.6)	−0.04	(0.2)	1.7	(0.3)	11.6	(1.9)
Oct 19	Control	29.0	(2.2)	−0.2	(0.3)	−6.6	(6.6)	6.8	(0.8)
	C_2H_2	35.5	(7.9)	−0.2	(0.02	6.4	(1.6)	12.3	(1.7)
	CH_3F	33.8	(12.0)	−1.0	(0.2)	−0.8	(1.8)	4.1	(1.1)

Means ± 1 standard error (S.E.) were measured prior to addition of nitrification inhibitors.

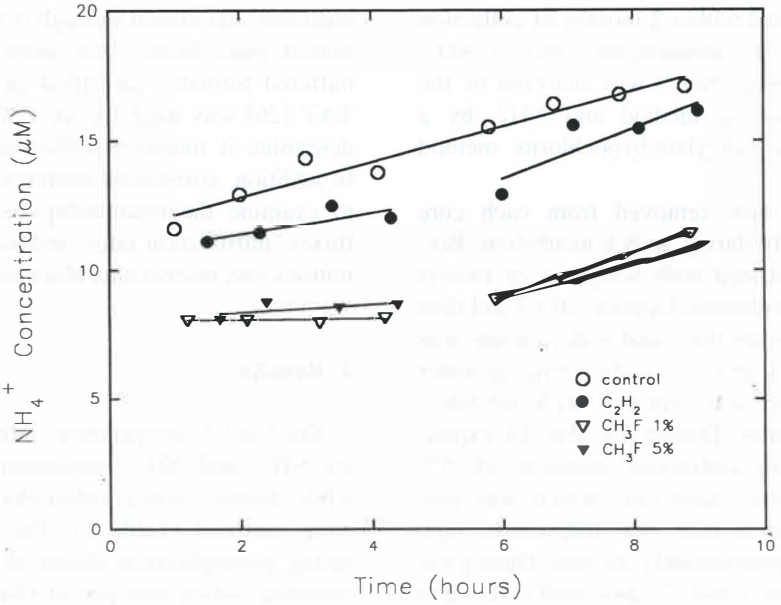

Fig. 1. NH_4^+ concentration (μM) in overlying water of cores during incubation period (h) on May 19, 1993.

were low in the fall. Sediment O_2 consumption increased from an average of 33 mmol O_2 m^{-2} d^{-1} on April 6 to 63 mmol O_2 m^{-2} d^{-1} on May 19, while NH_4^+ fluxes declined from 1.3 to 0.8 mmol N m^{-2} d^{-1} during this period (Table 3). There was no consistent pattern in the NO_3^- fluxes. Sediment O_2 consumption and NH_4^+ flux were extremely high in the control core on May 19 because of the presence

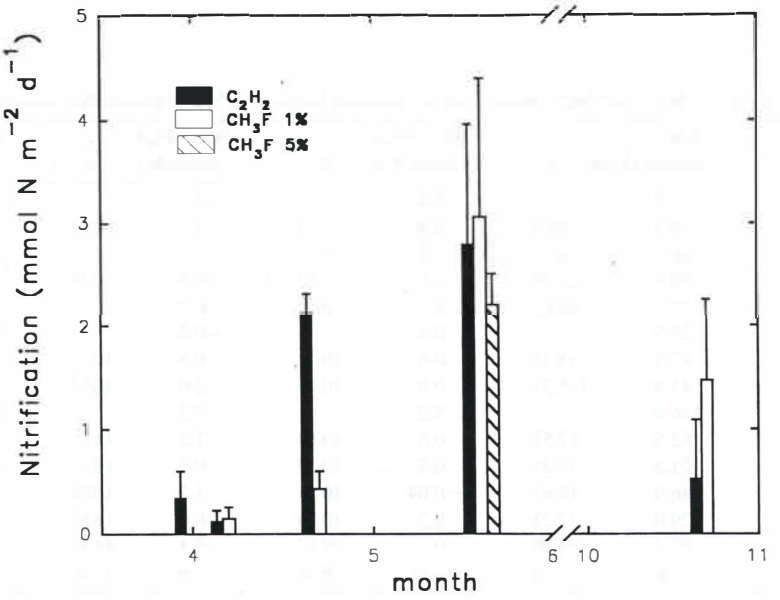

Fig. 2. Comparison of nitrification rates in San Francisco Bay sediment using C_2H_2 and CH_3F, nitrification inhibitors.

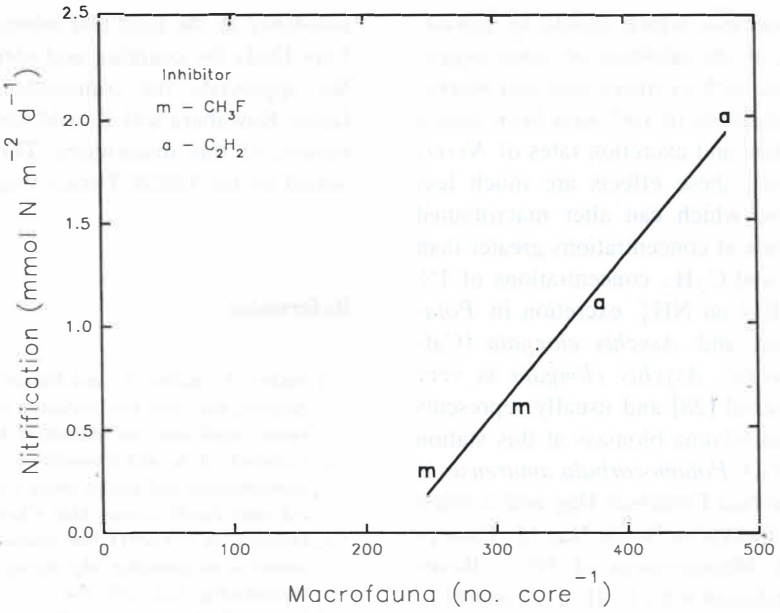

Fig. 3. Nitrification rate (mmol N m^{-2} d^{-1}) versus number of macrofauna in individual cores on April 22, 1993.

of a callianasid shrimp that excavated a burrow during the incubation period.

NH_4^+ flux out of the sediment increased after addition of either C_2H_2 or CH_3F to the overlying water (Fig. 1). Nitrification was minimal in early April, and peaked in May for all treatments (Fig. 2). Nitrification rates were not significantly different among the different treatments ($P = 0.59$), however, on April 22, rates measured with the C_2H_2 inhibition technique were about three times those measured using CH_3F. This difference may have been a result of differences in macrofauna biomass or number between the two treatments (Fig. 3, Table 3) with C_2H_2 cores having greater numbers and biomass of macrofauna than the CH_3F cores. In general, nitrification rates were positively correlated with macrofaunal biomass ($r = 0.39$, $P = 0.04$) and with sediment O_2 consumption ($r = 0.55$, $P = 0.002$). This suggests that macrofauna may control nitrification rates, and that NH_4^+ produced in the sediments is nitrified at a higher rate when macrofauna are abundant. In contrast, NH_4^+ will diffuse out of the sediments when there are fewer macrofauna. In addition, nitrification rates were negatively correlated with chlorophyll *a* ($r = -0.55$, $P = 0.003$) and phaeopigment concentrations ($r = -0.39$, $P = 0.04$). Sediment O_2 consumption and chlorophyll *a* were negatively correlated ($r = -0.39$, $P = 0.04$), while NH_4^+ fluxes were positively correlated with chlorophyll *a* ($r = 0.48$, $P = 0.009$) and phaeopigment ($r = 0.40$, $P = 0.04$).

4. Discussion

The results of these experiments suggest that CH_3F is as effective as C_2H_2 in inhibiting nitrification in intact sediment cores. Previous research [20,21] has shown that CH_3F is a more specific inhibitor than C_2H_2; that is, at high concentrations (> 1%), CH_3F has less effect than C_2H_2 on other microbial processes, such as methanogenesis and denitrification. The specificity of CH_3F could be used to distinguish between production of N_2O by nitrification or denitrification in sediments where N_2O fluxes are significant. We believe that CH_3F is an effective inhibitor of nitrification, based on the results of this study, and, in fact, may be superior to other inhibitors because of its specificity [20,21].

The central assumption which is made when using inhibitors is that the inhibitor only effects the enzyme of interest and has little or no effect on other

processes. Other processes which should be considered are the effects of the inhibitor on other organisms in the sediment, such as meiofauna and macrofauna. C_2H_2 concentrations of 10% have been shown to increase metabolism and excretion rates of *Nereis virens* [27]. However, these effects are much less than that of N-serve which can alter macrofaunal behavior and excretion at concentrations greater than 5 ppm [15]. CH_3F and C_2H_2 concentrations of 1% had a negligible effect on NH_4^+ excretion in *Potamocorbula amurensis* and *Asychis elongata* (Caffrey, unpublished data). *Asychis elongata* is very abundant in the channel [28] and usually represents over 50% of the macrofauna biomass at this station (Caffrey, unpublished). *Potamocorbula amurensis* is abundant throughout San Francisco Bay and is often the most abundant species in South Bay (J. Thompson, pers. comm.). Measurement of NH_4^+ fluxes before and after inhibition with C_2H_2 [14] or CH_3F (this study) has the benefit that each core functions as its own control. This is advantageous in systems with extreme spatial variability, or high densities of macrofauna.

These results also suggest that the supply of organic matter (as measured by sediment chlorophyll *a* and phaeopigment) is important in controlling nitrification rates, although there may be a lag in the response. Similar patterns of high nitrification in organic rich and bioturbated sediments have been observed in the Bering Sea [29], Danish coastal waters [30,31], the North Sea [32,33] and sediment microcosms [34]. Nitrification rates in South San Francisco Bay were highest in May, about a month after the spring bloom, while NH_4^+ fluxes peaked within 1–2 weeks of the spring bloom. This switch between high NH_4^+ flux early in the spring to high nitrification later in the spring may occur as the macrofauna respond to increased food supply by increasing rates of irrigation. Similar responses of macrofauna to organic inputs have been observed in Kiel Bight [35,36].

Acknowledgements

We thank Byron Richards, Scott Connard, Tracy Connell, Janet Guidetti, James Kuwabara, Francis Parchaso, Christy Tyler, and Sally Wienke for their assistance in the field and laboratory. We also thank Tom Duda for counting and identifying macrofauna. We appreciate the comments and suggestions of James Kuwabara and Ronald Oremland on an earlier version of this manuscript. This research was supported by the USGS Toxics Program.

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