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Ammonium addition inhibits ^{13}C-methane incorporation into methanotroph membrane lipids in a freshwater sediment

Stephen C. Nold [a,*], Henricus T.S. Boschker [b], Roel Pel [a], Hendrikus J. Laanbroek [a]

[a] *Center for Limnology, Netherlands Institute of Ecology, P.O. Box 1299, 3600 BG Maarsen, Netherlands*

[b] *Center for Estuarine and Coastal Ecology, Netherlands Institute of Ecology, P.O. Box 140, 4400 AC Yerseke, Netherlands*

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Abstract

To investigate the effect of ammonium addition on the species composition and activity of freshwater methane oxidizing bacteria, intact sediment cores were labeled with $^{13}CH_4$ and incubated under ambient and elevated ammonium concentrations. After 7 days, methanotroph activity was assessed by quantifying the isotopic composition of the carbon in membrane lipids. The 16-carbon rather than the 18-carbon methanotroph-specific biomarkers showed a clear enrichment in ^{13}C, suggesting the importance of group I methanotrophs in these sediments. Ammonium addition resulted in a depleted isotopic signal compared to ambient controls, suggesting that high ammonium concentrations inhibit methane incorporation into cellular components. These results compare favorably with studies that showed ammonium inhibition of methane oxidation, and extend these findings by demonstrating the effect of nitrogen fertilization on methanotroph lipid synthesis.

Keywords: Methane; Freshwater; Sediment; Ammonium; Lipid; Stable carbon isotope

1. Introduction

Methane-oxidizing bacteria and ammonia-oxidizing bacteria link the most reduced carbon and nitrogen compounds (CH_4, NH_4^+) to more oxidized forms (CO_2, NO_2^-). In addition to their usual source of energy, methanotrophs and ammonia oxidizers also utilize other substrates. Methanotrophs can oxidize ammonium and ammonia oxidizers can oxidize methane, yet neither type of organism can use this energy as the sole source for growth (reviewed by Bedard and Knowles [1]).

Although methanotrophs and ammonia oxidizers play an important role in global carbon and nitrogen cycles, their ecological interactions are not clearly understood. Numerous studies have shown that ammonium addition inhibits methane oxidation (e.g. [2–4]). However, methanotrophs may also oxidize the inhibiting ammonium, since ammonia oxidation was stimulated by enriching soil and freshwater sediments with methane [5–7]. Methanotrophs may even compete with ammonia oxidizers for reduced nitrogen when oxygen is plentiful [7] or under thermo-

* Corresponding author. Present address: Center for Microbial Ecology, 540 Plant and Soil Sciences Building, Michigan State University, East Lansing, MI 48824-1325, USA. Tel.: +1 (517) 353-7858; Fax: +1 (517) 353-2917; E-mail: nold@pilot.msu.edu

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philic conditions [8]. Methanotrophs and ammonia oxidizers may also compete for oxygen, since ammonia oxidation in a freshwater sediment was completely suppressed under high methane concentrations, presumably due to methanotroph oxygen consumption [7]. Due to these competitive interactions, ammonia oxidizers are not likely to contribute to methane oxidation in freshwater sediments [4,9].

Methanotrophs naturally fall into two groups (denoted I and II) based on differences in their internal membranes, phylogeny, carbon assimilation pathways, and fatty acid composition [10]. Group I methanotrophs primarily contain 16 carbon phospholipid fatty acids (PLFA, 16:0, 16:1) while group II methanotrophs contain monounsaturated 18 carbon PLFAs (18:1ω9c, 18:1ω8c) [11]. The fatty acids 16:1ω6c, 16:1ω5c, 16:1ω5t, and especially 16:1ω8c have been suggested as biomarker fatty acids for the group I methanotrophs, while the fatty acid 18:1ω8c is a highly specific signature for the *Methylosinus* and *Methylocystis* type II genera [11,12]. Ammonia-oxidizing bacteria primarily contain the fatty acids 16:0 and 16:1ω7c. These are common fatty acids that are also detected in group I methanotrophs [1].

A technique allowing the in situ association of microbial activity with microbial identity was recently developed. This method measures the extent of carbon isotope incorporation into specific membrane lipid biomarkers [8,13–15]. By labeling methanotrophs with $^{13}CH_4$ and analyzing the isotopic composition of individual PLFAs, Boschker et al. [13] identified the active methanotrophs in a freshwater sediment. To investigate the effect of ammonium addition on methanotrophic activity, we used this labeling technique to analyze sediment cores incubated with ambient and elevated ammonium concentrations. Our results indicate that ammonium addition (10 mM) results in a significant reduction in the carbon incorporated into methanotroph membrane lipids.

2. Materials and methods

2.1. Sampling

Freshwater sediment samples were collected on 22 October 1997 from Lake Loosdrecht, The Netherlands. Lake Loosdrecht is a shallow, eutrophic lake with peaty sediments characterized by high organic matter content ($>50\%$ by weight, [16]), seasonally high methane production rates ($>80\%$ of total anaerobic mineralization in autumn [17]), and high methane oxidation rates (ca. 75% of total sediment oxygen consumption [17]). Ammonium oxidation and aerobic mineralization also contribute to sediment oxygen consumption [18]. Due to continuous wind-driven mixing and shallow lake depths (mean depth = 1.8 m), the sediment surface remains oxygenated throughout the year [19].

Intact sediment cores were collected from under 1.7 m water using a hand-driven stainless steel corer. Individual cores were kept in the dark at in situ temperatures (10°C) after sampling and during laboratory incubations. Samples were collected from a small bay on the southeastern side of the island 'De Hoek' in the open lake (52°11′35″N, 5°3′44″E), a site with similar characteristics to the 'Marcus Pos' sampling site of Sinke et al. [18].

2.2. Incubations

The top 4 cm of sediment cores was transferred to diffusion chambers described by Sinke et al. [20] with an upper compartment containing the sediment and a lower compartment through which artificial porewater was pumped. The chambers were separated by a 0.02 μm aluminum oxide filter (Anodisc 47, Whatman International, Maidstone, UK) mounted between two 0.8-μm polyester filters (Versapor 800, Pall Gelman Sciences, Ann Arbor, MI) that allowed diffusion of dissolved gases and solutes into the sediment. An artificial porewater was designed to mimic natural ion concentrations [21] and contained: (all values given in mM) Ca^{2+}, 1.88; Mg^{2+}, 0.44; Fe^{2+}, 0.20; Na^{+}, 0.94; K^{+}, 0.06; NH_4^{+}, 1.00; HCO_3^{-}, 3.11; Cl^{-}, 1.13; PO_4^{2-}, 0.03. Oxygen concentrations in the artificial porewater were reduced to below 0.1 mg ml^{-1} by bubbling with helium before the porewater was anoxically transferred to gas sampling bags (Cali-5-Bond, Alltech Associates, Deerfield, IL). Thioglycolic acid was added as a reductant (0.55 mM) and Fe^{2+} was added after all oxygen was reduced. A methane gas headspace was introduced into the gas sampling bag and methane concentrations, measured daily in the artificial porewater, remained near satu-

ration during the entire experiment (1.40 ± 0.36 mM, mean ± S.D., $n = 21$). Ammonium concentrations were supplied as ammonium chloride at ambient (1 mM) and 10 times ambient concentrations (10 mM) to each of three replicate cores. The pH of the porewater delivered to the diffusion chambers was 7.2. Artificial porewater was pumped through the lower chamber at a rate of 0.05 ml min^{-1}, supplying ca. 50 times the sediment ammonium and methane demand, based on previously reported rates [20].

Stable carbon isotope compositions are reported in standard δ notation: $\delta^{13}C$ in parts per thousand (‰) = $((R_{sample}/R_{standard})-1)\times 1000$ where R is $^{13}C/^{12}C$. Carbon isotopic results are reported relative to the Vienna Peedee belemnite (VPDB) marine carbonate standard ($R_{VPDB} = 0.0112372$) [22]. In this notation, more positive values indicate a greater amount of ^{13}C.

All sediments were pre-incubated in the dark at 10°C for 7 days with non-labeled methane (−30‰, determined according to the method of Popp et al. [23]). After 1 week, the methane isotopic ratio was increased except in the non-labeled controls ($n = 3$) and all diffusion chambers were incubated for an additional 7 days. During this time, $\delta^{13}C$ values of the porewater methane increased logarithmically over 3 days, and remained within 1% of +1370‰ for the remaining 4 days. After incubation, sub-samples were collected from the top 0.5 cm of each core and stored at −20°C until further analysis. Sediment samples from this region contained the aerobic surface layer and the upper part of the anaerobic layer [20].

2.3. Lipid analyses

Membrane phospholipid fatty acids were prepared from sediment samples using a modified Bligh/Dyer lipid extraction procedure followed by silicic acid column chromatography purification and transmethylation by mild alkaline methanolysis according to the method of Guckert et al. [24]. The abundance and diversity of the resulting PLFAs were quantified using a gas chromatograph equipped with a flame ionization detector. PLFAs were identified by comparison of calculated equivalent chain length retention indices to published values and known standards [25]. Identification was verified by coupled gas chromatography-mass spectrometry analysis and by dimethyl disulfide derivatization to confirm monounsaturated double bond position [26]. Identifications were performed using two columns differing in polarity (50 m Ultra-2, 0.32 mm i.d., Hewlett Packard, and 50 m BPX-70, 0.33 mm i.d., SGE Austin, TX). Lipid nomenclature is as described by Guckert et al. [24].

Carbon isotopic composition of individual PLFAs was determined by gas chromatographic separation (BPX-70 column) followed by combustion to CO_2 in a Finnigan Type II combustion interface coupled to a Finnigan Delta-S isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany). Each peak was integrated over its entire width and corrected for background. Although baseline separation was achieved for most peaks, monounsaturated PLFAs were not cleanly separated and are reported as multiple fatty acids detected within one peak. For example, the $\delta^{13}C$ value reported for 16:1ω7c (peak 10 in Fig. 1) also contains the group I methanotroph biomarkers 16:1ω6c and 16:1ω8c. Since all chromatograms were similarly integrated, treatments can be readily compared, but the δ values reported for monounsaturated PLFAs should not be considered absolute.

2.4. Dimethyl disulfide (DMDS) derivatizations

To further separate monounsaturated PLFAs for isotopic determination, DMDS adducts were prepared according to the method of Nichols et al. [26]. This derivatization procedure replaces the double bonds in unsaturated fatty acids with methyl sulfide groups, effectively retarding the retention time of monounsaturated PLFAs and separating *cis* from *trans* isomers. Derivatization efficiency was greater than 98% when concentrations of derivatized and underivatized monounsaturated standards were compared by gas chromatography. Since the two methyl groups added during the derivatization procedure (−30‰) may differ in isotopic composition from the original fatty acid, $\delta^{13}C$ values of reported DMDS adducts were corrected for methyl group addition. After correction, DMDS adducts of derivatized standards exhibited a less than 1‰ difference in isotopic composition from underivatized standards (−0.52‰ ± 0.42, mean ± S.D., $n = 12$). Only one

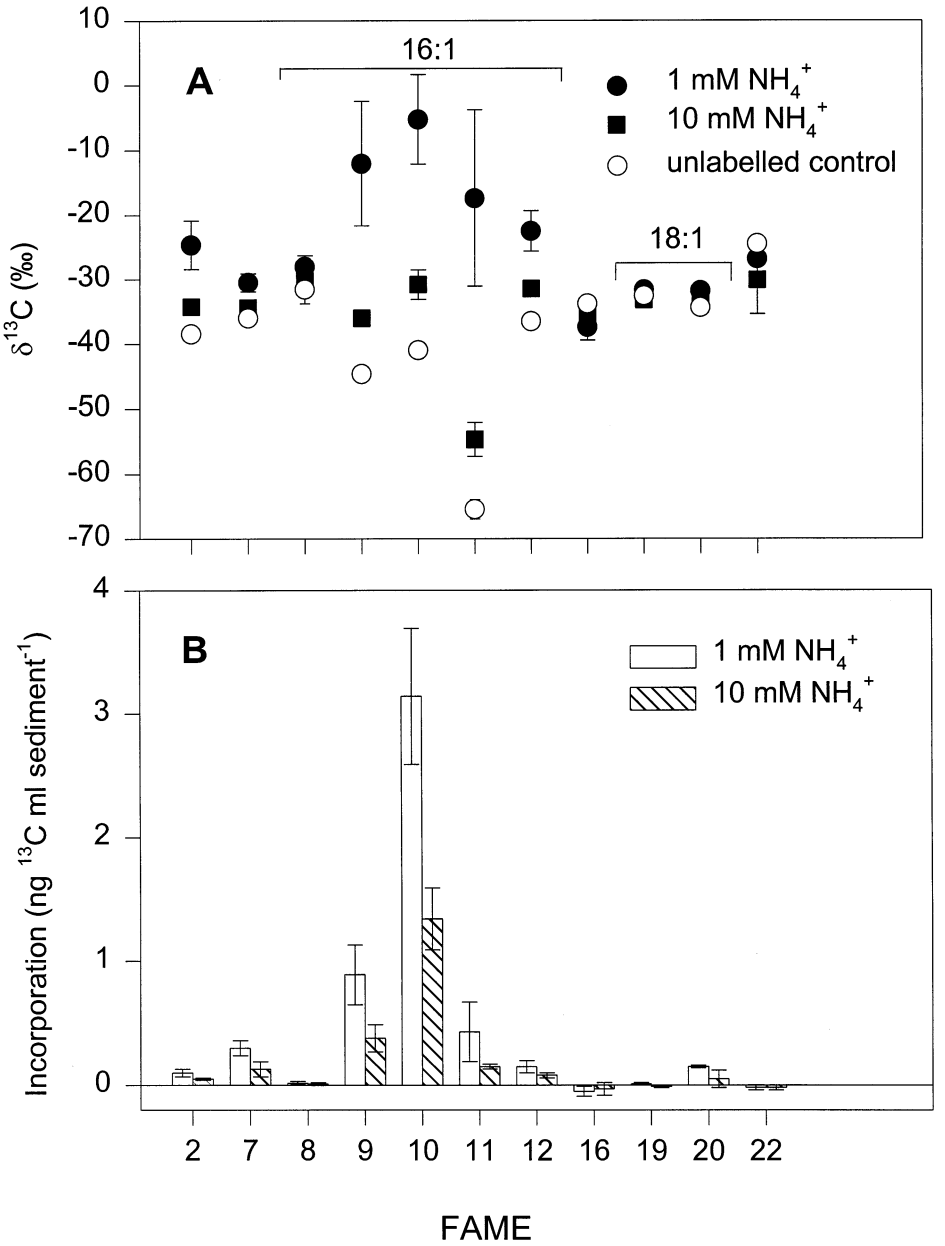

Fig. 1. Effect of ammonium addition on ^{13}C incorporated into the PLFAs of freshwater sediments. (A) Comparison of $\delta^{13}C$ values of PLFAs extracted from labeled sediments. (B) Total amount of ^{13}C incorporated into PLFAs. Values are means ± S.D. of three independent replicates. Numbers on the *x*-axis refer to selected PLFAs described in Table 1.

PLFA was successfully separated from the other monounsaturates using this method (16:1ω5t), and the reported concentration and $\delta^{13}C$ value of 16:1ω7c has been corrected for the absence of 16:1ω5t by a mass balance calculation.

2.5. Data analysis

$\delta^{13}C$ values for individual PLFAs were corrected for methyl group addition since the methanol used during the methanolysis procedure was relatively de-

pleted (−54‰). Incorporation of ^{13}C into PLFAs was calculated as: incorporation = $(F_e - F_c)[PLFA]_e$ where F is the fraction of ^{13}C in unlabeled controls (c) and experimental samples (e) after a 1-week incubation in the presence of $^{13}CH_4$ and PLFA concentrations are expressed as ng ^{13}C incorporated ml sediment^{-1}. F was calculated as $^{13}C/(^{13}C+^{12}C) = R/(R+1)$ where $R = (\delta^{13}C/1000+1)R_{VPDB}$ as defined above.

These data can be used to calculate the total methanotrophic biomass according to the formula:

$$[PLFA]_{methanotrophs} = \sum_{i=1}^{S} \frac{^{13}C_{ei} - ^{13}C_{ci}}{^{13}C_{CH_4}} \times [PLFA]_{ei} \quad (1)$$

where S is the total number of PLFA in the sample, ^{13}C is the mean $\delta^{13}C$ content of the unlabeled controls (c) and the experimental samples (e), $^{13}C_{CH_4}$ is the final $\delta^{13}C$ content of the labeled methane (+1370‰), and the PLFA concentrations in the experimental treatment are expressed in μg ml^{-1} sediment for the individual fatty acid, i.

This calculation requires the following assumptions. First, labeled methane must be rapidly incorporated by methanotrophs, leading to completely labeled methanotrophic biomass (balanced methanotrophic growth in forest soils was previously reported by Roslev et al. [27]). Second, methanotrophs must be the only microbes incorporating ^{13}C during the experiment.

3. Results

Before stable isotope analysis, we quantified PLFA diversity and abundance by standard gas chromatography using a flame ionization detector. This method cleanly separated the 16 and 18 carbon monounsaturated PLFAs. Although we readily detected group I methanotroph biomarkers (16:1ω8c, 16:1ω6c, 16:1ω5c and 16:1ω5t), we detected only trace amounts of the group II biomarker 18:1ω8c. Since resolving power is lost when the gas chromatograph is coupled to the mass spectrometer, the monounsaturated PLFA reported in Table 1 were not cleanly separated. The group I methanotrophic biomarkers 16:1ω8c and 16:1ω6c co-eluted with 16:1ω7c, a common PLFA. Likewise, the group II methanotrophic biomarker 18:1ω8c co-eluted with 18:1ω9c, another common PLFA.

Total phospholipid biomass in the Lake Loosdrecht sediments was 48.2 ± 19.4 and 68.0 ± 13.5 μg PLFA ml sediment^{-1} in cores incubated with ambient (1 mM) and elevated (10 mM) ammonium concentrations, respectively (mean ± SD, $n = 3$). These values are similar to those reported for other Dutch lake sediments [28] and Swedish boreal peatland soils [29]. Although PLFA concentrations were generally higher in the ammonium-treated sediment cores, the relative abundance and concentration of individual PLFAs were not significantly different between treatments (Table 1).

However, the carbon isotopic composition of individual PLFAs did vary, especially in fatty acids synthesized by methanotrophs and ammonia oxidizers (Table 1, Fig. 1A). The 16-carbon (group I) rather than the 18-carbon monounsaturated PLFAs (group II) showed a clear enrichment in isotopic signal when sediments were incubated with $^{13}CH_4$ at ambient ammonium concentrations (Fig. 1). Treatment with 10 mM ammonium resulted in a significantly depleted isotopic signal in the community PLFAs (Fig. 1).

Methanotrophic biomass can be estimated from the difference between the $^{13}CH_4$ incorporated by labeled sediment cores and unlabeled controls (Eq. 1). This calculation requires two assumptions. First, methanotroph doubling times must be significantly less than the duration of the experiment (i.e. the isotopic composition of methanotrophs should equal +1370‰ within 7 days). Second, the ^{13}C detected in PLFAs must be due to methanotrophic CH_4 incorporation rather than carbon recycling within autotrophic or heterotrophic portions of the community. Methanotrophic biomass from these calculations yielded 0.44 and 0.17 μg methanotroph PLFA ml sediment^{-1} for ambient and ammonium-treated samples, respectively. Compared to the total PLFA detected in Lake Loosdrecht sediments (Table 1), these values represent 0.91 and 0.24% of the total PLFA biomass in ambient and ammonium-treated samples, respectively.

Table 1
PLFA profiles and ^{13}C content of ammonium-treated Lake Loosdrecht sediments[a]

PLFA	PLFA number	1 mM NH_4^+		10 mM NH_4^+		Unlabeled control	
		Concentration[b]	$\delta^{13}C$	Concentration	$\delta^{13}C$	Concentration	$\delta^{13}C$
i14:0	1	0.51 (0.28)	−32.1 (0.9)	0.81 (0.23)	−33.7 (0.4)	0.42 (0.10)	−33.4 (1.0)
14:0	**2**	1.00 (0.50)	−24.6 (3.7)	1.43 (0.29)	−34.2 (0.9)	0.85 (0.16)	−38.5 (0.9)
i15:0	3	1.27 (0.65)	−31.1 (0.5)	1.85 (0.43)	−32.2 (0.5)	1.14 (0.17)	−32.6 (0.4)
a15:0	4	2.51 (1.33)	−30.4 (0.1)	3.76 (0.90)	−31.5 (0.2)	2.29 (0.36)	−31.3 (0.1)
15:0	5	0.51 (0.22)	−32.8 (1.6)	0.78 (0.19)	−35.1 (0.6)	0.47 (0.09)	−35.0 (1.1)
15:1ω6c/i16:0	6	0.75 (0.33)	−23.5 (1.3)	1.16 (0.28)	−34.9 (0.7)	0.72 (0.13)	−34.8 (0.3)
16:0	**7**	7.08 (2.90)	−30.5 (1.4)	10.29 (2.02)	−34.4 (0.8)	6.56 (1.19)	−36.0 (0.7)
16:1ω9t	**8**	0.56 (0.21)	−28.0 (1.7)	0.74 (0.12)	−30.1 (1.8)	0.52 (0.07)	−31.6 (2.3)
16:1ω9c[c]	**9**	3.49 (1.41)	−12.1 (9.6)	5.29 (1.16)	−36.0 (0.6)	3.35 (0.58)	−44.7 (0.4)
16:1ω7c[d]	**10**	10.07 (4.33)	−5.3 (6.9)	14.51 (3.12)	−30.8 (2.3)	9.24 (1.77)	−41.0 (1.3)
16:1ω5t[e]	**11**	1.10 (0.59)	−17.4 (13.6)	1.74 (0.35)	−54.7 (2.6)	1.08 (0.17)	−65.5 (1.5)
16:1ω5c/i17:0	**12**	1.40 (0.68)	−22.5 (3.1)	1.98 (0.43)	−31.5 (0.9)	1.36 (0.18)	−36.5 (0.8)
a17:0	13	0.42 (0.17)	−37.5 (1.5)	0.63 (0.14)	−34.8 (0.3)	0.41 (0.07)	−32.7 (2.4)
i17:1ω7c	14	0.58 (0.23)	−33.7 (2.1)	0.80 (0.18)	−30.3 (0.5)	0.53 (0.08)	−28.1 (1.1)
17:0	15	0.51 (0.19)	−32.7 (2.2)	0.75 (0.16)	−32.8 (0.4)	0.49 (0.08)	−30.9 (2.0)
17:1ω8c/Cy 17:0	**16**	1.36 (0.54)	−37.4 (2.0)	1.99 (0.44)	−35.6 (2.2)	1.28 (0.18)	−33.8 (0.5)
17:1ω6c	17	0.37 (0.17)	−36.7 (3.0)	0.54 (0.12)	−35.5 (1.7)	0.36 (0.06)	−32.0 (0.9)
18:0	18	1.06 (0.50)	−31.6 (0.2)	1.54 (0.28)	−33.3 (0.2)	1.06 (0.21)	−32.7 (0.3)
18:1ω9c	**19**	1.81 (0.71)	−31.6 (0.4)	2.39 (0.44)	−33.2 (0.5)	1.71 (0.27)	−32.6 (0.8)
18:1ω7c[f]	**20**	7.52 (2.78)	−31.8 (0.7)	10.39 (1.97)	−33.8 (0.8)	7.08 (1.18)	−34.4 (0.1)
18:2ω6	21	0.73 (0.33)	−25.0 (0.6)	0.95 (0.19)	−27.2 (0.1)	0.65 (0.16)	−24.7 (3.4)
Cy 19:0	**22**	0.84 (0.17)	−26.8 (2.3)	0.76 (0.43)	−30.1 (5.3)	0.78 (0.09)	−24.5 (0.9)
18:3ω3	23	1.03 (0.34)	−32.6 (0.2)	1.13 (0.22)	−32.8 (0.6)	0.83 (0.12)	−32.1 (0.4)
20:0	24	0.46 (0.15)	−33.5 (4.8)	0.49 (0.08)	−32.8 (0.7)	0.39 (0.02)	−31.7 (0.7)
18:4ω3	25	0.48 (0.11)	−30.9 (1.0)	0.54 (0.13)	−33.9 (0.4)	0.36 (0.07)	−31.7 (1.2)
20:4ω6	26	0.32 (0.10)	−23.9 (3.6)	0.32 (0.06)	−26.0 (1.1)	0.31 (0.06)	−27.3 (1.5)
20:5ω3	27	0.43 (0.11)	−28.4 (3.0)	0.41 (0.13)	−29.7 (1.2)	0.44 (0.10)	−28.9 (1.2)
Σ μg FAME ml sediment^{-1}		48.18 (19.38)		67.99 (13.49)		44.68 (7.72)	

[a]All values are means (standard deviations) of three independent replicates. Boldface denotes PLFA synthesized by methanotrophs and ammonia oxidizers.
[b]Concentrations are expressed in μg PLFA ml sediment^{-1}.
[c]Contains a large proportion of 10me16:0 and a smaller amount of 16:1ω7t.
[d]Contains a small proportion of 16:1ω8c and a smaller amount of 16:1ω6c.
[e]Data from DMDS-derivatized PLFA.
[f]Contains trace amounts of 18:1ω8c.

4. Discussion

Three lines of evidence suggest that group I methanotrophs are the dominant methane-oxidizing organisms in these freshwater sediments. First, we detected negligible amounts of the group II biomarker 18:1ω8c, whereas the group I biomarkers (16:1ω8c, 16:1ω6c, 16:1ω5c and 16:1ω5t) were readily detected, suggesting greater group I methanotrophic biomass. Second, after labeling with $^{13}CH_4$, 16-carbon PLFAs predominately received labeled methane rather than 18-carbon PLFAs, suggesting that group I methanotrophs actively incorporated methane into membrane lipids. Third, biogenic methane has a very depleted carbon isotopic signal (−65 to −50‰), and this low amount of ^{13}C is reflected in the methanotrophic biomass [30]. Those fatty acids that received the most label (16:1ω9c, 16:1ω7c, 16:1ω5t) were also the most depleted in the control incubations (Table 1, Fig. 1A, [13]), further suggesting the importance of group I methanotrophs in these sediments. The high abundance and activity of group I methanotrophs in Lake Loosdrecht compares favorably with other studies of freshwater methanotrophs

[10,31]. In contrast, Sundh et al. [29] found higher concentrations of type II methanotroph fatty acids in boreal peatland soils. Due to differences in cell size, calculated numbers of type I and type II methanotrophs were roughly similar and shared similar distribution with depth.

Since both methanotrophs and ammonia oxidizers contain the PLFAs 16:0 and 16:1ω7c [1,32], it is difficult to exclude the possibility that ammonia-oxidizing bacteria are incorporating $^{13}CH_4$ into membrane lipids. The fatty acid 16:1ω7c received the most label in this experiment, and this PLFA is common to both type I methanotrophs and ammonia oxidizers. However, incorporation of $^{13}CH_4$ into the major PLFA decreased equally when sediments were treated with 10 mM ammonium (16:0, 16:1ω9c, 16:1ω7c, and 16:1ω5t decreased by 43.3, 42.7, 42.8, and 34.7% of ambient controls, respectively). If ammonia-oxidizers contributed significantly to methane oxidation, we would expect differences the label incorporated into these PLFAs with ammonium treatment. Since all the major PLFAs decreased equally, this suggests that ammonia oxidizer PLFA do not significantly contribute to labeled lipid biomass.

The PLFA 16:1ω5t, as separated by DMDS derivatization, appears to be a highly specific fatty acid for identifying methanotrophic biomass and activity. This fatty acid displayed the lowest natural abundance and the greatest relative increase in isotopic composition after labeling (Table 1, Fig. 1A). 16:1ω5t was previously suggested as a signature biomarker for group I methanotrophs by Nichols et al. [33].

Methanotrophic biomass estimated from $^{13}CH_4$ incorporation yielded relatively low amounts ($<1\%$) compared to total microbial biomass. This result is unexpected given the high methane oxidation rates previously reported in Lake Loosdrecht sediments (ca. 10 mmol m^{-2} day^{-1}, which explains approximately 75% of the sediment oxygen consumption [20]). Our results indicate either that Lake Loosdrecht sediments contain a very small, but highly active, group of methanotrophs, or that one or more of our assumptions about methanotroph growth is incorrect. The assumption that $^{13}CH_4$ is the sole precursor for labeled methanotrophic biomass seems reasonable, although some respired $^{13}CO_2$ may act as a substrate for autotrophic growth. However, the assumption that the methanotrophs are growing rapidly and should therefore contain fully labeled carbon in their membrane lipids (+1370‰) may not be correct. If methanotrophic biomass was completely labeled, we would expect the lipid biomarkers to be much more enriched than the reported $\delta^{13}C$ values (<-5.3, Table 1). There may be several explanations for this observation. Isotopic discrimination during fatty acid synthesis was reported for methanotrophic bacteria [34]. This enzymatic discrimination would decrease the amount of labeled methane incorporated into PLFAs, resulting in a value lower than the expected +1370‰. However, these effects are negligible (−10‰ to −20‰) compared to the expected magnitude of change (+1370‰ minus −30‰ ($^{13}CH_4$ content in the control incubations) equals a +1400‰ expected increase in isotopic content). Alternatively, the limited labeling that occurred over the 7-day incubation may indicate that although these organisms are highly active [20], they may be growing very slowly (i.e. doubling times >7 days). Active, but slowly growing, microbial communities have been reported in other habitats (see citation [35] and references therein) and a similar physiological state may exist for freshwater sediment methanotrophs. The third and most likely explanation is that internal methane production by methanogenic *Archaea* may have diluted the labeled methane in the diffusion chamber, resulting in a lower $^{13}CH_4$ value than the expected +1370‰.

Although there are many reports of ammonium inhibiting methane oxidation, few studies have investigated the effect of nitrogen fertilization on the synthesis of cellular components. In a notable exception, Roslev et al. [27] showed a dramatic decrease (98%) in the amount of radiolabeled methane incorporated by methanotrophs when soils were treated with 1 μmol ammonium gfw $soil^{-1}$. These authors also observed decreased carbon conversion efficiencies in ammonium-treated incubations, leading them to conclude that methane and ammonium metabolism most likely took place in the same methanotrophic organism [27]. We detected a significant reduction in the amount of $^{13}CH_4$ incorporated into methanotroph lipids in our ammonium-treated cores. These results clearly demonstrate the effect of ammonium addition on methanotroph activity (i.e. membrane synthesis).

However, it is not possible to infer from these data the role of methanotrophs as ammonia oxidizers in Lake Loosdrecht sediments.

These findings demonstrate the application of a new lipid labeling technique to investigate the effect of environmental manipulation on freshwater microorganisms. The use of DMDS adducts provided additional resolution when separating monounsaturated PLFA for isotopic determination. Furthermore, these results extend previous process rate measurements by directly assessing the effect of ammonia addition on methanotroph carbon metabolism.

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