Article

Net sediment production of methane, distribution of methanogens and methane-oxidizing bacteria, and utilization of methane-derived carbon in an arctic lake

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Abstract

Our study illustrates that methanogenesis and methane oxidation within the sediments of a small arctic lake are spatially variable, and using an integrated set of approaches, strongly suggests that fine-scale patterns of spatial variability in distribution of methane oxidizing bacteria (MOB) and methanogens are related to the nature of bioturbation and utilization of MOB by *Chironomus* larvae. Greater net sediment methane production occurred at a lake depth where concentrations of both methanogen and MOB DNA in the sediments were higher. The ratios of MOB/methanogen DNA on tubes and in the sediment supported the hypothesis of microbial gardening of MOB only at the lake depth where net methanogenesis was relatively high. *Chironomus* hindguts contained higher concentration of methanogen DNA and showed a trend toward higher concentration of MOB DNA compared to foreguts. The underlying mechanism for differential distribution of methanogen and MOB DNA across the gut needs further investigation, but the pattern suggests that the relationship between *Chironomus* larvae, methanogens, and MOB is more complex than simply feeding on and assimilation of MOB as may be implied by low $\delta^{13}C$ of larvae. Vertical distribution into the sediment profile of methanogens and MOB DNA reflects the oxygen regime of the overlying water and was consistent with reports of *Chironomus* bioturbation activities on particle distribution within the sediment profile.

Key words: arctic lake, *Chironomus*, *Chironomus* tubes, methane oxidizing bacteria (MOB) DNA, methane-derived carbon, methanogen DNA, net sediment methane production, sediment profile

Introduction

Methane is the most atmospherically abundant hydrocarbon with >20-fold greater radiative effectiveness per molecule than carbon dioxide (Caldwell et al. 2008). Biogenic methane is produced by methanogenic bacteria as an end product of anaerobic respiration (Werne et al. 2002). In aquatic habitats, methane diffuses upward from the sediment to the water column and atmosphere (Whalen 2005). Such methane emissions from lakes represent 6–16% of total identified atmospheric sources (Bastviken et al. 2004).

Methane may be consumed by methane oxidizing bacteria (MOB) in the sediment and water column. MOBs are the only known organisms that use methane as a direct carbon source (Hanson and Hanson 1996). Methane oxidation serves an important role in controlling methane release into the atmosphere (Whalen 2005) and is responsible for reducing up to 20% of the net atmospheric methane flux (Valentine and Reeburgh 2000).

Oxidation of methane is also a way to recycle methane-derived carbon (MDC) into food webs (see review Jones and Grey 2011). Macroinvertebrate communities that consume MOB obtain energy from a novel

carbon source compared to photosynthetically derived carbon (Bunn and Boon 1993, Grey et al. 2004, Jones et al. 2008). Stable isotope analysis has been used to infer use of MDC into food webs in many aquatic ecosystems (Jones and Grey 2011). Reported $\delta^{13}C$ values for biogenic methane generally range from −50 to −110‰, reflecting isotopic fractionation during methanogenesis (Whiticar 1999) and further fractionation during methane oxidation (Coleman et al. 1981, Whiticar and Farber 1986). The low $\delta^{13}C$ value of MDC results in a low $\delta^{13}C$ value for organisms such as *Chironomus* that consume MOB. Chironomid species, especially *Chironomus* spp, have been reported with $\delta^{13}C$ values ranging from −38 to −75‰ (e.g., Grey et al. 2004, Eller et al. 2005, 2007, Hershey et al. 2006).

Some aquatic invertebrates, notably chironomid larvae, have been reported to feed on MOB (Deines et al. 2007). Most species of chironomid larvae live in tubes constructed from silk and debris. In lake sediments, chironomid larval tubes and associated behaviors permit larvae to obtain oxygen and flush waste products, which increases oxygen concentration within tubes compared to surrounding sediments (Stief et al. 2005). Such bioturbation behavior has been hypothesized to promote growth of MOB on the tubes and facilitate use of MOB by the larvae, a phenomenon that has been referred to "microbial gardening" (Eller et al. 2005, Deines et al. 2007, 2009, Jones et al. 2008). Larval chironomids in streams are also known to garden diatoms on their tubes (Pringle 1985, Hershey et al. 1988). The microenvironment created by larvae increases MOB growth and supply to larvae (Eller et al. 2007). If such gardening occurs in lake sediments, it should be reflected in a greater ratio of MOB, but not methanogens, on tubes than in sediments. Furthermore, a high rate of assimilation of MOB or methanogens would be expected to result in a decline in their concentration within the gut during gut passage.

Previous comparative studies have found that $\delta^{13}C$ of larval Chironomini, especially *Chironomus*, is lower in lakes with low dissolved oxygen, suggesting that use of MDC is greater under hypoxic conditions (Hershey et al. 2006, Jones et al. 2008). Furthermore, across benthic invertebrate taxa studied in small arctic lakes, $\delta^{13}C$ values were lower in offshore areas below the stratification depth, compared to nearshore areas where overlying water was better oxygenated, indicating within-lake variation in use of MDC (Hershey et al. 2006). In this study, we measured net sediment production of methane and *Chironomus* $\delta^{13}C$ at 2 depths in a small arctic lake and characterized the distributions of methanogen and MOB DNA in sediments and bottom water on *Chironomus* tubes, and in larval gut contents at the same depths. We use the expression net sediment production of methane to reflect that considerable methane oxidation occurs at the sediment–water interface (Whalen 2005), such that the rate of methane released to the water column is the difference between gross production in the sediments and oxidation at the sediment water interface. We hypothesized that (1) greater net sediment production of methane occurs in deeper lake sediments, where bottom water is hypoxic, compared to shallower sediments, resulting in greater methane availability for MOB and greater utilization of MDC by *Chironomus*; (2) variation in methanogen and MOB DNA concentrations in sediments and on tubes corresponds to variation in rates of net sediment methane production at different depths in the lake and is reflected in *Chironomus* $\delta^{13}C$; (3) methanogen and MOB communities found on tubes are consistent with MOB gardening by *Chironomus*, such that the ratio of MOB/methanogen concentrations will be greater on tubes than within the sediment profile; and (4) lower concentrations of methanogen and MOB DNA in larval hindguts as compared to the foreguts reflects assimilation of foregut microbial flora.

Methods

The study was conducted during July 2009 in the vicinity of the Toolik Lake Field Station (68°38′N, 149°43′W) in the northern foothills of the Brooks Mountain Range in arctic Alaska (http://toolik.alaska.edu/). We focused on *Chironomus* larvae from Lake GTH 112 (68°40′N, 149°14′W), which has a surface area of 0.025 km^2, max depth of ~5.6 m, and an average depth ~2.1 m. This oligotrophic lake is intermittently stratified and supports high densities of *Chironomus* (Northington et al. 2010), which have low $\delta^{13}C$ values relative to photosynthetically derived sources and to many other lakes in the region (Hershey et al. 2006). This study focused on sediments from the approximate mean and maximum depths (2 and 5 m, respectively). On all sampling dates, the lake was stratified. Overlying water was well oxygenated at 2 m (8.6–12.0 mg L^{-1}) and hypoxic at 5 m (0.8–0.9 mg L^{-1}).

Six intact cores each were collected with a KB corer (4.5 cm internal diameter) from 2 and 5 m from Lake GTH 112 for analysis of net sediment methane production. Cores were extruded into 25 cm long, 4.5 cm internal diameter incubation cores to achieve a sediment core height of ~12 cm, with ~13 cm of the overlying water. Cores were sealed at the bottom with acrylic bottoms with o-rings seals, fitted with floating stirbars, and capped with acrylic tops with o-ring seals. Internally, the caps had central bevels leading to sampling port fittings guarded by septa. Sealed cores were arranged around a central shaft which supported magnets that rotated at 1 rpm to turn stirbars (modified from Gettel et al. 2007). This apparatus was designed to prevent establishment

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of chemical stratification and maintain a gas–water equilibrium within the cores without disturbing the sediment–water interface.

Cores were incubated at 8 °C. Overlying water from each core (3 mL) was sampled initially and at 12 and 24 h through the sampling port with a syringe, and injected into 30 mL preevacuated, He-filled serum vials fixed with 0.1 mL of 1 N HCl. Headspace water in the core was replaced using lake water from the depths of core collection. Methane equilibrated into the headspace of the serum vial was quantified with a Shimadzu GC8A flame ionization detection gas chromatograph using a carrier of ultrahigh purity nitrogen (N_2) at a flow rate of 33 mL min^{-1} and a 1 m molecular sieve 5A column. Net sediment methane production was calculated as the time-linear rate of CH_4 accumulation in cores. These measurements represent net sediment methane production because there was no inhibition of methane oxidation during the experiment.

Three sediment cores each from 2 and 5 m were collected for analyses of methanogen and MOB DNA. A 10 mL sample of overlying water from each core was filtered through a 25 mm glass fiber filter (Whatman GFC). Filters were stored in CTAB buffer (Schaefer 1997). Sediments were sampled at the sediment–water interface (0 mm), and cores were sectioned to sample at 1, 2, 5, 10, and 60 mm into the sediment profile (down-profile). For each layer of each core, small samples were placed in preweighed tubes with 1 mL of CTAB.

Chironomus larvae and tubes were collected from 2 and 5 m using an Eckman dredge for analyses of methanogen and MOB DNA. Larval tubes were gently picked from the sediment surface at the top of the dredge with forceps and placed into a glass scintillation vial filled with lake water. Most tubes were abandoned by larvae during collection. Larval tubes were returned to the field station, gently prodded to remove any larvae that had not vacated tubes, and stored individually in 1 mL of CTAB. Eight tubes were analyzed from each depth. Dredged sediment was sieved through a 450 μm mesh net in the field to collect larvae for methanogen and MOB DNA analyses. Larvae and coarse sediments were transported to the lab for sorting. *Chironomus* larvae were dissected under a dissecting microscope to remove foregut and hindgut contents. Assimilation and absorption occur in the midgut (Breznak 1982), but it was not practical to separate the gut into 3 sections. We separated the gut into 2 sections because we expected that differences in methanogen and MOB DNA due to gut passage would be seen by comparing foregut and hindgut contents, even if both groups of samples contained midgut portions. Foregut and hindgut contents were stored in CTAB buffer. This process was repeated for 8 samples of foreguts and hindguts from larvae from both depths.

Additional *Chironomus* larvae were collected from 2 and 5 m using an Eckman dredge and processed for stable isotope analyses. *Chironomus* larvae were held overnight in filtered lake water, placed into microcentrifuge tubes and dried in a 60 °C oven for 3 d. Dried larvae were homogenized and crushed with a mortar and pestle. Subsamples (1 mg) were analyzed for $\delta^{13}C$ by the Stable Isotope Laboratory at the University of California-Davis.

Quantification of methanogen and MOB DNA

A variety of methods including fluorescence *in situ* hybridization, denaturing gradient gel electrophoresis, restriction fragment length polymorphism and polymerase chain reaction (PCR) have been used to study and identify methanogens (e.g., Wright and Pimm 2003, Eller et al. 2005, Yu et al. 2005). A methanogenic specific gene, *mcrA*, which has been the target of several studies (e.g., Eller et al. 2005, Juottonen et al. 2006), encodes the α-subunit of methyl-coenzyme M reductase, which aids in methane formation. However, because methanogens have a diverse phylogeny, it has been difficult to identify a single primer set to target all methanogens (Juottonen et al. 2006), so we explored several primer sets.

The oxidation of methane is catalyzed by either soluble or particulate forms of methane monooxygenase (Hanson and Hanson 1996). Under natural conditions, all MOB express a membrane-bound enzyme, particulate methane monooxygenase (pMMO). MOB have been studied mainly using the pMMO gene, *pmoA* (Tavormina et al. 2008). The *pmoA* gene encodes for the α-subunit of pMMO, which is central to aerobic methanotrophy and is highly conserved within the bacterial domain (Hanson and Hanson 1996), is present in almost all known MOB (Holmes et al. 1995), and was targeted in this study.

Sediment, filters, and gut content samples were processed to extract and amplify DNA using CTAB DNA extraction (Schaefer 1997). Sample DNA was quantified and checked for purity using a Thermo Scientific Nanodrop Spectrophotometer. Initial PCR reactions were run on a Cepheid Smart Cycler1 to test for primers and conditions that would optimize results. After several test runs, methanogenic and MOB specific primer sets A189/mb661 and Met86/Met1340 (Table 1) were determined to deliver consistent results. Test runs were also conducted to determine optimal dilutions of extracted DNA for consistent results.

Once primer sets, conditions, and dilutions were determined, subsequent PCR runs were done using an Applied Biosystems StepOne real-time PCR System using 48 well plates. Each reaction consisted of 10 μL of Power Sybr Green PCR Master Mix, 1 μL of each 10 μM forward and reverse primer, 8 μL of sterile DI water, and 1 μL of

Table 1. PCR forward (top) and reverse (bottom) primer pairs used in PCR analyses.

Primer Pair	Sequence	Tm (°C)	Target	Reference
Met 86 F Met 1340R	GCTCAGTAACACGTGG GGTGTGTGCAAGGAG	46.3	Methanogen	Wright and Pimm 2003
A189 mb661	GGNGACTGGGACTTCTGG CCGGMGCAACGTCYTTACC	56.2	MOB	Costello and Lindstrom 1999

template. Each plate included 3 negative controls, samples run in triplicate, and 3 concentrations of standards also run in triplicate. When using primer set A189/mb661, the standard was genomic DNA from *Methylococcus capsulatus* (ATCC catalog item number 19069D-5); when using primer set Met86/Met1340, the standard was genomic DNA from *Methanosarcina acetivorans* Strain C2A (ATCC catalog item number 35395D-5). Standards were set up in dilution series of 0.5 ng μL^{-1} DNA, 0.05 ng μL^{-1} DNA, and 0.005 ng μL^{-1} DNA. The PCR run consisted of (1) an initial activation step of 95 °C for 15 min; (2) 40 cycles of 95 °C for 15 s, 55 °C for 30 s, 72 °C for 1 min, and 80 °C for 15 s, during which fluorescence was measured; and (3) a melt curve. After the real-time PCR was completed, PCR results (ng DNA μL^{-1} of extract) were converted to ng DNA g^{-1} sample (or mL^{-1} for water samples) for comparison.

Data analysis

For all statistical analyses, p-values ≤ 0.05 were considered significant, and $0.05 < p < 0.1$ were considered suggestive of a difference between groups (Ramsey and Shafter 2002). Net rates of sediment methane production were compared between depths with a t-test on ln (x + 28) transformed data because 28 was the smallest integer that would remove negative values from the dataset because some cores showed a net consumption rather than net production of methane. Negative net sediment production of methane implies that methane oxidation in the cores exceeded that produced in the sediments, which could have occurred through oxidation of methane that was already present in water overlying the sediments in the cores. One of the 5 m cores was a significant outlier based on Grubbs' test for single outliers (Grubbs 1969) and was dropped from the dataset prior to analyses. The underlying cause of the outlier effect is unknown but likely reflects disturbance of the core during collection, transport, or extrusion.

Methanogen and MOB DNA concentrations in overlying water from sediment cores were each compared between depths with t-tests on ln (x+1) transformed data. Methanogen and MOB DNA concentrations from sediment cores were each analyzed using 2-way ANOVAs on ln (x+1) transformed data to investigate effects of lake depth, distance down-profile, and the interaction between lake depth and down-profile distance. With only 2 lake depths, there were no additional pairwise comparisons following significant depth main effects. Significant and suggestive down-profile and interaction effects were followed by one-way ANOVAs to examine the main effect of distance down-profile on methanogen and MOB DNA concentration at each lake depth. However, no post hoc tests between pairs of distances within the profiles were performed because we had no *a priori* hypotheses regarding specific pairwise comparisons (e.g., 5 vs. 10 mm down-profile). We examined down-profile patterns graphically and evaluated those patterns. Tube methanogen and MOB DNA concentrations were compared between depths using t-tests on ln (x+1) transformed data. Methanogen and MOB DNA concentrations are not directly comparable to each other because their genome copy numbers are highly variable (Hildenbrand et al. 2011). To examine gut processing of methanogen and MOB DNA and evaluate how gut contents and processing differ depending on lake conditions, gut contents were analyzed using 2-way ANOVA on ln (x+1) transformed data to evaluate effects of gut region and lake depth. Significant main effects were followed by t-tests to evaluate differences in methanogen and MOB DNA concentrations between gut regions at each depth.

We evaluated whether microbial communities on larval tubes supported the hypothesis of microbial gardening by comparing the ratio of MOB DNA/methanogen DNA on tubes compared to the same ratio within the sediment profile. MOB DNA concentrations on tubes versus sediments are not directly comparable because tube mass and sediment mass, which are incorporated into the DNA concentration data, are not comparable to each other, but the ratios of MOB DNA/methanogen DNA are independent of sample mass. A higher ratio of MOB DNA/methanogen DNA on tubes compared to that within the sediment profile would provide evidence for gardening of MOB. We compared the tube ratio to the ratio at each depth into the sediment profile (tube ratio vs.

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ratios at 1, 2, 5, 10, and 60 mm) using a sign test (Hollander and Wolf 1999).

Chironomus larvae $\delta^{13}C$ values were compared between 2 and 5 m with a t-test. We did not use a mixing model to calculate % MDC for larvae because such models need to be corrected for fractionation. Although fractionation is often reported as −20‰ (see Jones and Grey 2011), it varies widely with environmental conditions (Coleman et al. 1981, Whiticar and Farber 1986), which were different between our 2 sites. Therefore, we were concerned that mixing models would not provide us with a valid comparison of use of MDC between sites.

Results

Net sediment production of methane

The mean (±SE) net sediment production of methane at 2 m in Lake GTH 112 was 18.4 ± 12.9 μmol CH_4 m^{-2} d^{-1} (Table 2). Net sediment methane production was significantly greater, 896.1 ± 392.8 μmol CH_4 m^{-2} d^{-1}, at 5 m ($p = 0.009$).

Methanogen and MOB DNA concentrations in sediments and overlying water

Methanogen DNA concentration in overlying core water was highly variable and did not differ significantly between 2 and 5 m depths ($p < 0.3$). MOB DNA concentration in overlying core water was >10-fold greater at 5 m than at 2 m ($p = 0.02$; Table 3).

Table 2. Mean ± SE net production of methane in cores from 2 and 5 m depths in Lake GTH 112 based on n = 6 and n = 5 cores, respectively. Statistical analyses were based on ln (x + 28) transformed values (see text for explanation).

Depth	Net methane production (mean μmol m^{-2} d^{-1})	SE	t-value	p-value
2 m	18.4	12.9	3.31	0.009
5 m	896.1	392.8		

Overall sediment concentration of methanogen DNA was greater in the 5 m than in the 2 m cores ($p < 0.0001$; Table 4; Fig 1a). Methanogen DNA concentration also varied significantly with distance down-profile ($p < 0.004$; Table 4). The interaction of depth and distance down-profile was suggestive of significance ($p < 0.07$), which likely reflected the different patterns down-profile at the 2 depths (Fig. 1a). At 2 m, methanogen DNA concentrations did not differ significantly among distances down-profile ($p < 0.4$), although estimated peak concentration occurred 10 mm into the sediments. At 5 m, however, methanogen DNA concentrations varied significantly among distances down-profile ($p = 0.002$), with a large peak concentration occurring at 2 mm down-profile, and lowest concentration at the sediment surface (Fig. 1a).

Concentration of MOB DNA was also greater in the 5 m cores than in the 2 m cores ($p = 0.002$; Table 4), and the pattern was very similar to that of methanogen DNA (Fig 1a and 1b). Averaged across depth, variation in MOB DNA concentration was only suggestive of a down-profile effect ($p = 0.05$; Table 4), and the interaction of lake depth and distance down-profile was not significant ($p = 0.3$). At 2 m, MOB concentrations did not differ with depth down-profile ($p = 0.4$). At 5 m, the effect of distance down-profile was suggestive of an effect ($p = 0.06$), with highest concentrations at 1 and 2 mm down-profile.

Larval tube and gut methanogen and MOB DNA concentrations

Methanogen DNA concentrations on *Chironomus* tubes were 3.3 ± 1.6 and 16.3 ± 9.4 ng DNA g^{-1} tube (mean ± SE) at 2 m and 5 m, respectively (Fig. 2). MOB DNA concentrations on tubes were 3.8 ± 1.5 and 31.4 ± 19.5 ng DNA g^{-1} tube (mean ± SE) at 2 m and 5 m, respectively. Despite the difference in magnitude between means, methanogens and MOB concentrations on tubes were highly variable and not significantly different between lake depths (methanogen DNA: $t = 1.57$, $df = 13$, $p < 0.2$; MOB DNA: $t = 1.46$, $df = 14$, $p < 0.2$; Fig 2).

Comparison of MOB DNA/methanogen DNA ratio on tubes to that at various distances down-profile showed that at 2 m, the ratio on tubes was 2.1 ± 0.39. The same ratio down-profile at 2 m was variable and not consistently

Table 3. Mean ± SE concentrations of methanogen and MOB DNA in overlying core water from 2 and 5 m depths in Lake GTH 112. Each mean is based on n = 3 cores from the respective depth. Statistical analyses were based on ln (x +1) transformed values.

	2 m	5 m	t-value	p-value
Methanogen DNA (ng L^{-1})	0.040 ± 0.016	3.05 ± 2.72	1.36	0.24
MOB DNA (ng L^{-1})	0.77 ± 0.39	8.06 ± 2.6	3.55	0.02

Table 4. Summary of statistical results for analyses of ln transformed methanogen and MOB DNA concentrations down-profile in cores from GTH 112. Analyses were based on 6 vertical sections from 3 cores collected at both 2 m and 5 m depths. Significant 2-way ANOVAs were followed by one-way ANOVAs to examine variability in methanogen and MOB concentrations down-profile. Fewer degrees of freedom for methanogen compared to MOB DNA concentrations reflect one missing value for the sediment surface in one core.

	F-ratio	df	p-value
Methanogen DNA 2-way model	5.62	11, 33	0.0002
Distance down-profile	4.85	5, 23	0.004
Lake depth	24.06	1, 23	<0.0001
Distance down-profile*depth	2.43	5, 23	0.07
Methanogen 1-way ANOVAs			
Distance down-profile 2 m	1.27	5, 11	0.3
Distance down-profile 5 m	7.58	5, 12	0.002
MOB DNA 2-way model	2.79	11, 24	0.02
Distance down-profile	2.59	5, 24	0.05
Lake depth	12.04	1, 24	0.002
Distance down-profile*depth	1.22	5, 24	0.3
MOB 1-way ANOVAs			
Distance down-profile 2 m	1.14	5, 12	0.4
Distance down-profile 5 m	2.90	5, 12	0.06

different than the ratio on the tubes (Table 5). At 5 m, the MOB/methanogen DNA ratio on tubes was 1.5 ± 0.41, which was significantly greater than the sediment ratio down-profile (Table 5, $p = 0.03$).

Gut content analysis revealed that methanogen and MOB DNA were present in both gut regions at both depths but varied across gut region and depth (methanogen DNA: $F_{3,28} = 4.94$, $p = 0.007$; MOB DNA: $F_{3,27} = 3.63$, $p < 0.03$; Fig. 3). Methanogen DNA concentration was greater in hindguts compared to foreguts ($F_{1,28} = 4.56$, $p = 0.04$) and greater at 5 m than at 2 m ($F_{1,28} = 6.52$, $p = 0.02$), but the interaction between gut region and depth was only suggestive of significance ($F_{1,28} = 3.75$, $p = 0.06$). At 2 m, methanogen DNA concentration was not significantly different between gut regions ($t = 0.58$, $df = 14$, $p = 0.6$). At 5 m, mean methanogen DNA in hindguts was nearly 7-fold greater than that in foreguts, but the difference was only suggestive of significance ($t = 2.076$, $df = 14$, $p = 0.06$). Although the overall ANOVA for MOB DNA concentrations was significant, main effects of depth ($F_{1,27} = 3.91$, $p = 0.06$), gut region ($F_{1,27} = 3.24$, 0.08), and depth by gut region interaction ($F_{1,27} = 3.48$, $p = 0.07$) were only suggestive of significant patterns. MOB concentrations were very similar in foreguts and hindguts at 2 m (Fig. 3), but at 5 m, mean MOB concentrations were ~10-fold higher in hindguts, although the pattern was suggestive of significance ($F_{1,14} = 3.66$, $p = 0.08$).

Chironomus $\delta^{13}C$

Larvae at 5 m had a significantly lower $\delta^{13}C$ value (−35.59 ± 0.10‰) than those at 2 m (−35.04 ± 0.06‰; $t = 4.7$, $df = 4$, $p = 0.009$; Table 6), although the magnitude of the difference was small. *Chironomus* $\delta^{13}C$ values were more similar to methane $\delta^{13}C$ than to that of photosynthetic sources (Table 6) and were generally lower than those of other benthic invertebrate consumers in GTH 112 and other area lakes (Hershey et al. 2006). Photosynthetic sources ranged from −26.5 to −30.2‰ (Hershey et al. 2005; Table 6). Methane $\delta^{13}C$ measured during 2007 and 2010 was −41.7 and −33.6‰, respectively (Table 6).

Discussion

Our results generally agree with expected depth-specific patterns of net sediment production of methane, methanogen, and MOB DNA abundance in sediments and on tubes, and *Chironomus* $\delta^{13}C$. Furthermore, spatial variation of methanogen and MOB DNA within the sediment profile provides insights into larval habitat and foraging behavior and methane biogeochemistry. However, methanogen and MOB DNA patterns in larval foreguts and hindguts were not as expected and illustrate that the role of methanogens and MDC in *Chironomus* ecology is not fully understood.

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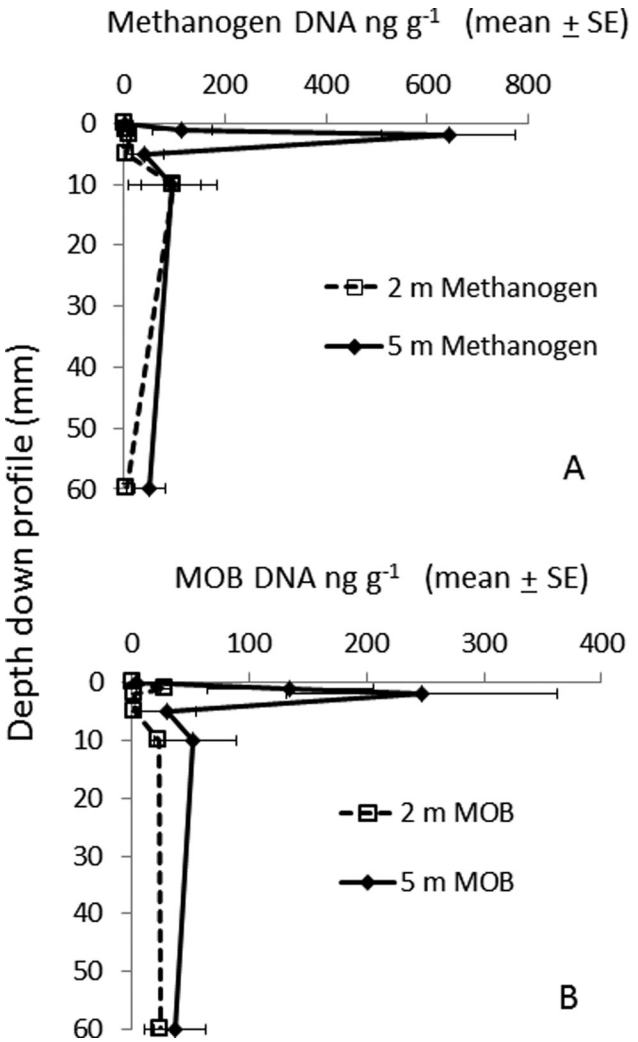

Fig. 1. Methanogen (panel A) and MOB (panel B) DNA concentrations at discrete distances down-profile based on 1 mm sections of cores from 2 m and 5 m in Lake GTH 112. For each depth, n = 3 cores. For each section in each core, values represent the mean of typically 3 determinations per section (range = 2 to 6 determinations per section). See text for statistical results.

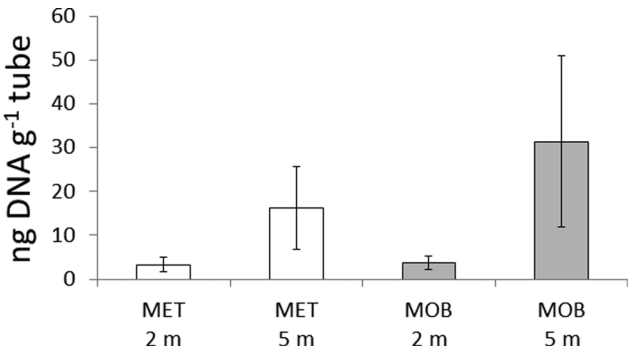

Fig. 2. Methanogen and MOB DNA concentrations (mean ± SE ng DNA g^{-1} tube) on *Chironomus* larval tubes at 2 and 5 m from Lake GTH 112 (n = 8 tubes per depth). Means did not differ significantly (see text).

Net sediment production of methane in Lake GTH 112

Greater net sediment production of methane at 5 m compared to 2 m reflects the pattern of dissolved oxygen stratification in the lake. Lake GTH 112 stratifies intermittently and rapidly develops an hypoxic hypolimnion (Hershey et al. 2006 and unpubl. data). During the summer season when samples were obtained, the oxygen profiles showed that bottom water was hypoxic at 5 m (0.8–0.9 mg L^{-1}). Oxygen penetrates deeper into sediments when dissolved oxygen is higher in the overlying water (Kajan and Frenzel 1999). Thus, deeper oxygen penetration into the sediment likely explains the lower methanogen abundance and the lower rate of net sediment methane production at 2 m compared to 5 m, similar to other studies that found greater methane efflux from sediments at hypoxic or anoxic sites compared to oxic sites (Zeicus and Winfrey 1976, Frenzel et al. 1990, Liikanen and Martikainen 2003, Eller et al. 2005).

Our estimates of net sediment production of methane of 18 and 896 µmol CH_4 m^{-2} d^{-1} from intact lake cores are comparable to other estimates in the literature. Estimated sediment diffusive flux was 50–200 µmol CH_4 m^{-2} d^{-1} in oligotrophic Lake Stechlin (Casper et al. 2003), 196–311 µmol CH_4 m^{-2} d^{-1} in mesoeutrophic Lake Washington (Kuivila et al. 1988), 1264–7900 CH_4 µmol m^{-2} d^{-1} in eutrophic Lake Kevätön (Liikanen and Martikainen 2003), and ranged from 28 to 6563 µmol CH_4 m^{-2} d^{-1} in 6 Finnish lakes spanning mesotrophic to eutrophic conditions (Huttunen et al. 2006). Frenzel et al. (1990) found fluxes of 35 and 480 µmol CH_4 m^{-2} d^{-1} from sediments with oxic and hypoxic overlying water, respectively, in sediments from Lake Constance, which was undergoing reoligotrophication. Hypereutrophic Onondaga Lake averaged 5600–12 700 µmol CH_4 m^{-2} d^{-1} based on a 16-year study (Matthews et al. 2005). These studies report highest rates in hypereutrophic lakes, but show considerable overlap in sediment methane efflux across lake types and further illustrate importance of oxygen in the overlying water for this process.

Our estimates of net sediment methane production cannot be compared to estimates of methane emissions to the atmosphere from whole lakes, which range from 0 to 20 g C m^{-2} yr^{-1} (providing a mean daily rate of 0–4566 µmol CH_4-C m^{-2} d^{-1}; St. Louis et al. 2000, Bastviken et al. 2004) because different processes are incorporated into whole lake versus core incubation studies. In a study of 1

Table 5. Ratio of MOB/methanogen DNA concentration on *Chironomus* tubes (mean ± SE) compared to the same ratio at various distances down-profile (ratios calculated from data presented in Figs. 1 and 2). Mean ± SE for methanogens and MOB DNA concentrations at each depth, from which ratios were derived, are given in Figure 1.

MOB DNA/methanogen DNA ratios							
Depth	Tube	1 mm	2 mm	5 mm	10 mm	60 mm	Sign test
2 m	2.1 ± 0.39	4.4	0.3	0.8	0.2	5.4	ns
5 m	1.5 ± 0.41	1.2	0.4	0.7	0.6	0.7	p = 0.03

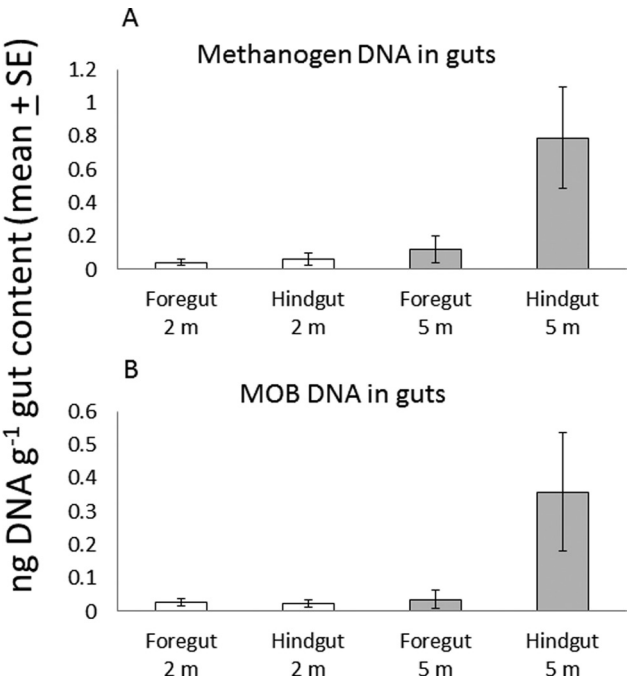

Fig. 3. Methanogen (panel A) and MOB (panel B) DNA concentrations (mean ± SE ng DNA g^{-1} gut content) in *Chironomus* larval guts at 2 and 5 m from Lake GTH 112 (n = 8 larvae at each depth, but only 7 foreguts at 2 m). See text for statistical comparisons.

lake and 2 reservoirs, Huttunen et al. (2006) found that sediment methane flux was not closely related to whole lake emission due to both ebullition and water column oxidation, which can consume much or all of sediment methane production (Bastviken et al. 2003). In boreal reservoirs and north temperate lakes, methane oxidation in the water column was estimated as 26–90% and 62–79%, respectively, of methane production (Striegl and Michmerhuizen 1998, Venkiteswaran and Schiff 2005). Rudd and Hamilton (1978) estimated that approximately 67% of methane produced over an annual cycle in Lake 227 was oxidized in the water column. Due to the shallow overlying water in our cores (approximately 13 cm, which is not dissimilar to what might occur in a wetland), oxidation in the experimental cores may have been in the range of that reported for wetlands (Whalen 2005). In the lake, the potential for methane oxidation would be much greater because oxidation would occur throughout the entire water column in Lake GTH 112. Furthermore, our core method did not capture ebullition; ebullition is clearly the major process in methane emissions from lakes (Casper et al. 2000, Walter et al. 2006) and has been incorporated into many of the whole lake estimates (Bastviken et al. 2004).

Net sediment production of methane was much lower at 2 m compared to 5 m. We considered whether a difference in methane oxidation between sites could account for the observed difference. Bastviken et al. (2008) noted that in shallow water, proportionally more of the methane produced escaped oxidation due to greater turbulence and less opportunity for MOB activity within the water column. In our in laboratory experiments, cores from both depths were exposed to the same conditions with respect to turbulence and water depth, such that turbulence and water depth effects on methane escapement were not mimicked. Sediments may have been better oxygenated initially in the 2 m cores (we did not measure

Table 6. $\delta^{13}C$ values (mean ± SE) for larval *Chironomus* from Lake GTH 112 and for potential organic matter source materials.

Component	$\delta^{13}C$ mean (‰)	S. E.	N
Chironomus 2 m	−35.04	0.06	3
Chironomus 5 m	−35.59	0.10	3
Surficial sediment	−27.1	0.4	5
Periphyton†	−28.1	0.6	19
Seston†	−30.2	0.2	8
Terrestrial plants†	−26.5	0.5	6
CH_4 GTH 112‡	−41.7, −33.6		2
CH_4 survey‡	−44.3	2.6	24

† Values from Hershey et al. 2004.
‡ $\delta^{13}C$ CH_4 from GTH 112 in 2007 and 2010, respectively, and from 24 lakes in the region in June-July 2007 (Hershey unpubl. data).

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oxygen within the sediment profile or in core water), and the overlying water from the lake was clearly better oxygenated at 2 m, conditions which would permit greater methane oxidation. However, concentrations of MOB DNA were considerably higher in the overlying water and within the sediment profile in 5 m cores compared to 2 m cores. Although DNA concentration is not necessarily proportional to methanogenic or MOB activity, these results are consistent with a pattern of greater methane production and oxidation at the 5 m site, such that the difference in gross methane production between sites was likely larger than the difference between net production.

Methanogen and MOB DNA in sediments and on tubes

Because methanogens are strict anaerobes, distribution of methanogens and MOB are not generally expected to be spatially coincident (Jones et al. 1987, Thauer and Shima 2006). At the 2 m depth, although there was no significant down-profile pattern for either methanogen or MOB concentration, the peak concentration of methanogen DNA that we observed occurred deeper in the sediment profile (10 mm) than did peak concentration of MOB DNA (1 mm; Fig. 1), which would be expected based on segregated spatial distributions for the respective microbes. Furthermore, the peak of MOB nearer to the sediment–water interface is also in agreement with the expectation of aerobic conditions near the sediment surface because the overlying water was well oxygenated at 2 m. Thus, the low methanogen DNA concentrations that occurred above 10 mm down-profile at 2 m likely reflected oxic conditions in the first few mm down-profile.

In contrast, concentrations of methanogen and MOB DNA were strongly stratified down-profile at 5 m but with coinciding peak concentrations from 1 to 2 mm. This pattern of coinciding peaks is not consistent with the expected segregated distributions of anaerobic and aerobic organisms; however, spatial co-occurrence of methanogens and MOB has been reported previously (Kajan and Frenzel 1999, Deines et al. 2007). Methanogens can survive periods of oxic conditions (Roslev and King 1994, Peters and Conrad 1995). Kajan and Frenzel (1999), working in saturated rice patty soils, suggested that vertical coincidence of these microbes in sediments, such as we observed at 5 m lake depth, may be a result of bioturbation due to irrigation and feeding activity of chironomid larvae. Experimental studies of the impact of *Chironomus* on particle distribution showed that *Chironomus* redistributed particles vertically within the sediments, although the effect was restricted to the immediate vicinity of their burrows (Matisoff and Wang 2000). Furthermore, particle redistribution by *Chironomus* was largely lateral rather than vertical in the first few mm down-profile (Matisoff and Wang 2000). Lateral redistribution in the first few mm down-profile could lead to coincident peak abundances of methanogen and MOB, such as we observed in 5 m cores. Furthermore, vertical redistribution at deeper distances down-profile could be the underlying mechanism for the relatively homogenous distributions of methanogen and MOB DNA further down-profile at 5 m. The contrasting pattern in 2 m cores of segregated distributions of methanogen and MOB DNA may reflect that oxygen conditions were never favorable for methanogens at 1–2 mm down-profile, such that there were few methanogens present to be redistributed. However, additional research is needed to fully understand the mechanisms affecting distribution of methanogens.

For consumers such as *Chironomus* that construct tubes, bioturbation is believed to promote conditions favorable for both respiration and feeding (Pinder 1986, Eller et al. 2007, Jones et al. 2008) and, potentially, also for growth of MOB (Deines et al. 2007). Although peak MOB concentration down-profile occurred at 1–2 mm at both depths, MOB were clearly detectable at 10 and 60 mm down-profile, where sediments would have been anaerobic (Whalen unpubl. data). Because *Chironomus* tubes may extend several cm into the sediment profile, oxic microsites needed for MOB may develop from larval irrigation activities (Jones et al. 2008, Kajan and Frenzel 1999), which can draw solutes, including oxygen, through tubes into the sediment from the overlying water (Matisoff and Wang 1998).

The growth of MOB on larval tubes that results from bioturbation behavior has been considered microbial gardening (Deines et al. 2007, 2009). At 5 m, the significantly greater ratio of MOB/methanogen DNA on tubes compared to that in the sediment supports the hypothesis that bioturbation by *Chironomus* enhances the microenvironment of the tube to the benefit of MOB despite hypoxic or anoxic conditions in the surrounding sediments. Thus, bioturbation may serve to reduce oxygen limitation of MOB at 5 m. At 2 m, where the water column was well oxygenated, the MOB/methanogen DNA ratio was variable down-profile and not consistently lower than the ratio on the tubes (Table 5), suggesting that gardening was not important. Thus, the importance of gardening may be spatially variable and dependent on oxygen conditions and site-specific methane production.

Methanogen and MOB DNA in Chironomus guts

Higher concentration of methanogen DNA in *Chironomus* hindguts compared to foreguts was an unexpected finding of our study. Eller et al. (2007) found that methanogens were not an important component of chironomid diets.

Our findings do not dispute those of Eller et al. (2007), but they do suggest the need for further investigation into the relationship between methanogens in chironomid diets and their presence in larval guts. It is possible that *Chironomus* harbor methanogens in the hindgut, as occurs in termites (Breznak 1982, Ohkuma et al. 1999, Gomathi et al. 2009). Our study does not address potential symbiosis between *Chironomus* and methanogens, and previous studies utilizing fluorescence *in situ* hybridization analyses of thin sections of *Chironomus plumosus* larval tissue found no evidence of a symbiotic relationship between chironomid larvae and methanogens (Eller et al. 2007). However, *Chironomus* larval guts are anoxic (Deines et al. 2007). A parsimonius explanation for higher concentration of methanogens in *Chironomus* hindguts compared to foreguts is that the gut environment enhances methanogen growth by providing anaerobic conditions and a source of nutrients. This latter hypothesis requires further investigation.

In addition to a higher methanogen DNA concentration in *Chironomus* larval hindguts, there was a suggestive trend toward higher concentration of MOB DNA in the 5 m larval hindguts compared to foreguts, which may be indicative of MOB activity within the gut. MOB are expected to be active where both methane and oxygen are present (Kajan and Frenzel 1999, Thauer and Shima 2006), implying that these microbes may have been obtaining oxygen in the gut. Studies in termites have determined that methane oxidation does not occur in the hindguts (Pester et al. 2007); however, unlike *Chironomus*, termites do not have hemoglobin. The higher concentration of MOB DNA in 5 m larval hindguts compared to foreguts leads us to hypothesize that methane oxidation may be occurring, possibly supported by oxygen diffusion across the gut membrane, even if the interior of the gut is anoxic. Mechanistic studies are also needed to test this hypothesis. Thus, our results suggest that the relationship between *Chironomus* larvae, methanogens, and MOB is more complex than simple utilization of ingested MOB by larvae.

Chironomus $\delta^{13}C$ and use of MDC

Organisms that use MDC are ^{13}C depleted compared to particulate organic matter sources derived from photosynthesis. In general, $\delta^{13}C$ increases by approximately 0.5–1‰ between food source and consumer (Peterson and Fry 1987). Methane $\delta^{13}C$ in GTH 112 was relatively enriched compared to values reported in the literature, which generally range from −40 to −110‰ (e.g., Kohzu et al. 2004, Jones et al. 2008). Our estimates of methane $\delta^{13}C$ varied more than 8‰ and did not include depth specific samples from the date when larvae were collected for this study. Methane $\delta^{13}C$ may also differ between the epiliminion and hypolimnion due to fractionation during oxidation within the water column (Bastviken et al. 2003). Thus, although *Chironomus* larvae from GTH 112 were not as ^{13}C depleted as those reported in some other studies (e.g., Deines et al. 2007), the role of MDC may still be quite important because CH_4 in this lake was not as ^{13}C depleted as reported in most other studies. The isotopic signature of larvae from 2 m suggests that these larvae used slightly less MDC than *Chironomus* larvae at 5 m, although the difference in $\delta^{13}C$ was small. However, potential differences in fractionation during oxidation between 2 m compared to 5 m affect our ability to infer the relative importance of MDC to larvae from the two depths. Although the proportional contribution of MDC to *Chironomus* diets cannot be estimated, *Chironomus* $\delta^{13}C$ values from both depths were more similar to methane $\delta^{13}C$ than to photosynthetic sources. These values strongly suggest an important role for methane-derived carbon in larval nutrition, which is consistent with the presence of methanogen and MOB DNA in their environment, on their tubes, and in their gut contents.

Conclusions

This study of a small arctic lake shows that greater net sediment methane production occurs at lake depths where concentrations of both methanogen and MOB DNA in the sediments are higher. The ratios of MOB/methanogen DNA on tubes and in the sediment support the hypothesis of microbial gardening of MOB only at the lake depth where net methanogenesis was relatively high. Vertical distribution into the sediment profile of methanogens and MOB DNA reflects the oxygen regime of the overlying water and is consistent with reports of *Chironomus* bioturbation activities on particle distribution within the sediment profile. Thus, our study illustrates that methanogenesis and methane oxidation within lake sediments are spatially variable, and using an integrated set of approaches, strongly suggests that fine-scale patterns of spatial variability in distribution of MOB and methanogens are related to the nature of bioturbation and utilization of MOB by *Chironomus* larvae.

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References

Bastviken D, Cole JJ, Pace ML, Tranvik L. 2004. Methane emissions from lakes: Dependence of lake characteristics, two regional assessments, and a global estimate. Glob Biogeochem Cy. 26:261–320.

Bastviken, D, Cole JJ, Pace ML, Van de Bogert C. 2008. Fates of methane from different lake habitats: Connecting whole-lake budgets and CH_4 emissions. J Geophys Res. 113, G02024, doi:10.1029/2007JG000608.

Bastviken D, Ejlertsson J, Sundh I, Tranvik L. 2003. Methane as a source of carbon and energy for lake pelagic food webs. Ecology. 84:969–981.

Breznak, JA. 1982. Intestinal microbiota of termites and other xylophagous insects. Ann Rev Microbiol. 36:323–343.

Bunn SE, Boon PI. 1993. What sources of organic-carbon drive food webs in billabongs - a study based on stable-isotope analysis. Oecologia. 96:85–94.

Caldwell SL, Laidler JR, Brewer EA, Eberly JO, Sandborgh SC, Colwell FS. 2008. Anaerobic oxidation of methane: Mechanisms, bioenergetics, and the ecology of associated microorganisms. Environ Sci Technol. 42:6791–6799.

Casper P, Furtado A, Adams DD. 2003. Biogeochemistry and diffuse fluxes of greenhouse gases (methane and carbon dioxide) and dinitrogen from the sediments in oligotrophic Lake Stechlin. In: Doschel R, Adams DD, editors. Lake Stechlin: an approach to understand an oligotrophic lowland lake. Arch Hydrobiol – Adv Limnol. 58:53–71.

Casper P, Maberly SC, Hall GH, Finlay BJ. 2000. Fluxes of mthane and carbon dioxide from a small productive lake to the atmosphere. Biogeochemistry. 49:1–19.

Coleman DD, Risati JB, Schoell M. 1981. Fractionation of carbon and hydrogen isotopes by methane-oxidizing bacteria. Geochem Cosmochem Acta. 45:1033–1037.

Costello AM, Lindstrom ME. 1999. Molecular characterization of functional and phylogenic genes from natural populations of methanotrophs in lake sediments. Appl Environ Microbiol. 65:5066–5074.

Deines P, Bodelier PLE, Eller G. 2007. Methane-derived carbon flows through methane-oxidizing bacteria to higher trophic levels in aquatic systems. Environ Microbiol. 9:1126–1134.

Deines P, Wooller M J, Grey J. 2009. Unraveling complexities in benthic food webs using a dual stable isotope (hydrogen and carbon) approach. Freshwater Biol. 59:2243–2251.

Eller G, Deines P, Grey J, Richnow H, Kruger M. 2005. Methane cycling in lake sediments and its influence on chironomid larval $\delta^{13}C$. FEMS Microbiol Ecol. 54:339–350.

Eller G, Deines P, Kruger M. 2007. Possible sources of methane-derived carbon for chironomid larvae. Aquat Microb Ecol. 46:283–293.

Frenzel P, Thebrath B, Conrad R. 1990. Oxidation of methane in the oxic surface layer of a deep lake sediment (Lake Constance). FEMS Microbiol Lett. 73:149–158.

Gettel, GM, Giblin AE, Howarth RW. 2007. The effects of grazing by the snail, *Lymnaea elodes*, on benthic N_2 fixation and primary production in oligotrophic, arctic lakes. Limnol Oceanogr. 52:2398–2409.

Gomathi V, Ramasamy K, Reddy MRVP, Ramalakshmi A, Ramanathan A. 2009. Methane emission by gut symbionts of termites. Acad J Plant Sci. 2:189–194.

Grey J, Kelly A, Jones RI. 2004. High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. Limnol Oceanogr. 49:239–244.

Grubbs, F. 1969. Procedures for detecting outlying observations in samples. Technometrics. 11:1–21.

Hanson RS, Hanson TE. 1996. Methanotrophic bacteria. Microbiol Rev. 60:439–471.

Hershey AE, Beaty S, Fortino K, Kelly S, Keyse M, Luecke C, O'Brien WJ. 2005. $\delta^{13}C$ signatures of chironomids in arctic lakes: role and direction of benthic-pelagic coupling. Verh Internat Verein Limnol. 29(1):292–295.

Hershey AE, Beaty S, Fortino K, Kelly S, Keyse M, Luecke C, O'Brien WJ, Whalen SC. 2006. Stable isotope signatures of benthic invertebrates in arctic lakes indicate limited coupling to pelagic production. Limnol Oceanogr. 51:177–188.

Hershey AE, Hiltner AL, Hullar MAJ, Miller MC, Vestal JR, Lock MA, Rundle S, Peterson BJ. 1988. Nutrient influence on a stream grazer: *Orthocladius* microcommunities respond to nutrient input. Ecology. 69:1383–1392.

Hildenbrand C, Stock T, Lange C, Rother M, Soppa J. 2011. Genome copy numbers and gene conversion in methanogenic Archaea. J Bacteriol. 193:734–743.

Holmes A, Costell A, Lidstrom M, Murrell J. 1995. Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionary related. FEMS Microbiol Lett. 132:203–208.

Hollander M, Wolf DA. 1999. Nonparametric statistical methods. Wiley. 787 p.

Huttunen, JT, Välsänen TS, Hellsten SK, Martikainen PJ. 2006. Methane fluxes at the sediment-water interface in some boreal lakes and reservoirs. Boreal Environ Res. 11:27–34.

Jones RI, Carter CE, Kelly A, Ward S, Kelly DJ, Grey J. 2008. Widespread contribution of methane-cycle bacteria to the diets of lake profundal chironomid larvae. Ecology. 89:857–864.

Jones RI, Grey J. 2011. Biogenic methane in freshwater food webs. Freshwater Biol. 56:213–229.

Jones WJ, Nagle DP Jr, Whitman WB. 1987. Methanogens and the diversity of archaebacteria. Microbiol Rev. 51:135–177.

Juottonen H, Galand PE, Yrjala K. 2006. Detection of methanogenic Archaea in peat: a comparison of PCR primers targeting the mcrA gene. Res Microbiol. 157:914–921.

Kajan R, Frenzel P. 1999. The effect of chironomid larvae on production, oxidation and fluxes of methane in a flooded rice soil. FEMS Microbiol Ecol. 28:121–129.

Kohzu A, Kato C, Iwata T, Kishi D, Murakami M, Nakano S, Wada E. 2004. Stream food web fueled by methane-derived carbon. Aquat Microb Ecol. 36:189–194.

Kuivila KM, Murray JW, Devol AH, Lindstrom ME, Reimers CE. 1988. Methane cycling in the sediments of Lake Washington. Limnol Oceanogr. 33:571–581.

Liikanen A, Martikainen PJ. 2003. Effects of ammonium and oxygen on methane and nitrous oxide fluxes across sediment-water interface in a eutrophic lake. Chemosphere. 52:1287–1293.

Matisoff G, Wang X. 1998. Solute transport in sediments by freshwater infaunal bioirrigators. Limnol Oceanogr. 43(7):1487–1499.

Matisoff G, Wang X. 2000. Particle mixing by freshwater infaunal bioturbators: Midges (Chironomidae: Diptera) and mayflies (Ephermeridae: Ephemeroptera). J Great Lakes Res. 26(2):174–182.

Matthews DA, Effler SW, Mathews CM. 2005. Long-term trends in methane flux from the sediments of Onondaga Lake, NY: Sediment diagenesis and impacts on dissolved oxygen resources. Arch Hydrobiol. 163:435–462.

Northington RM, Keyse MD, Beaty SR, Whalen SC, and Hershey AE. 2010. Benthic Secondary production in eight oligotrophic arctic Alaskan lakes. J N Am Benthol Soc. 29(2):465–479.

Ohkuma M, Noda S, Kudo T. 1999. Phylogenic relationships of symbiotic methanogens in diverse termites. FEMS Microbiol Lett. 171:147–153.

Pester M, Tholen A, Freidrich MW, Brune A. 2007. Methane oxidation in termite hindguts: absence of evidence and evidence of absence. Appl Environ Microbiol. 2007:2024–2028.

Peters V, Conrad R. 1995. Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. Appl Environ Microbiol. 61:1673–1676.

Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. Ann Rev Ecol System. 18:293–320.

Pinder LCV. 1986. Biology of freshwater Chironomidae. Ann Rev Entomol. 31:1–23.

Pringle CM. 1985. Effects of chironomid (Insecta: Diptera) tube building activities on stream diatom communities. J Phycol. 21:185–194.

Ramsey FL, Schafter DW. 2002. The Statistical Sleuth. 2nd edition. California: Duxbury- Thomson Learning.

Roslev P, King GM. 1994. Survival and recovery of methanotrophic bacteria under oxic and anoxic conditions. Appl Environ Microbiol. 60:2602–2608.

Rudd JWM, Hamilton RD. 1978. Methane cycling in a eutrophic shield lake and its effects on whole lake metabolism. Limnol Oceanogr. 23:337–348.

Schaefer EF. 1997. A DNA assay to detect the toxic dinoflagellate *Pfiesteria piscicida*, and the application of a PCR based probe [master's thesis]. [Greensboro (NC)]: University of North Carolina.

St. Louis VL, Kelly CA, Duchemin E, Rudd JWM, Rosenberg DM. 2000. Reservoir surfaces as sources of greenhouse gases to the atmosphere: a global estimate. Bioscience. 50:766–775.

Stief P, Nazarova L, De Beer D. 2005. Chimney construction by *Chironomus riparius* larvae in response to hypoxia: microbial implications for freshwater sediments. N Am Benthol Soc. 24:858–871.

Striegl RG, Michmerhuizen CM. 1998. Hydrologic influence on methane and carbon dioxide dynamics at two north-central Minnesota lakes. Limnol Oceanogr. 43:1519–1529.

Tavormina PL, Ussler III W, Orphan VJ. 2008. Planktonic and sediment- associated aerobic methanotrophs in two seep systems along the North American Margin. Appl Environ Microbiol. 74:3985–3995.

Thauer RK, Shima S. 2006. Methane and microbes. Nature. 440:878–879

Valentine DL, Reeburgh WS. 2000. New perspectives on anaerobic methane oxidation. Environ Microbiol. 2:477–484.

Venkiteswaran JJ, Schiff SL. 2005. Methane oxidation: isotopic enrichment factors in freshwater boreal reservoirs. Appl Geochem. 20:683–690.

Walter KM, Zimov SA, Chaton JP, Verbyla D, Chapin FS III. 2006. Methane bubbling from Siberian thaw lakes as a positive feedback to climate warming. Nature. 443:71–75.

Werne JP, Baas M, Sinninghe Damste JS. 2002. Molecular isotopic tracing of carbon flow and trophic relationships in a methane-supported benthic microbial community. Limnol Oceanogr. 47:1694–1701.

Whalen SC. 2005. Biogeochemistry of methane exchange between natural wetlands and the atmosphere. Environ Eng Sci. 22:73–94.

Whiticar MJ. 1999. Carbon and hydrogen isotope systematic of bacterial formation and oxidation of methane. Chem Geol. 161:291–314.

Whiticar MJ, Faber E. 1986. Methane oxidation in sediment and water column environments – isotope evidence. Org Geochem. 10:759–768.

Wright AG, Pimm C. 2003. Improved strategy for presumptive identification of methanogens using 16S riboprinting. J Microbiol Meth. 55:337–349.

Yu Y, Lee C, Kim J, Hwang S. 2005. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol Bioeng. 89:670–679.

Zeikus JG, Winfrey MR. 1976. Temperature limitation of methanogenesis in aquatic sediments. Appl Environ Microbiol. 31:99–107.

 DOI: 10.5268/IW-2.2.416