

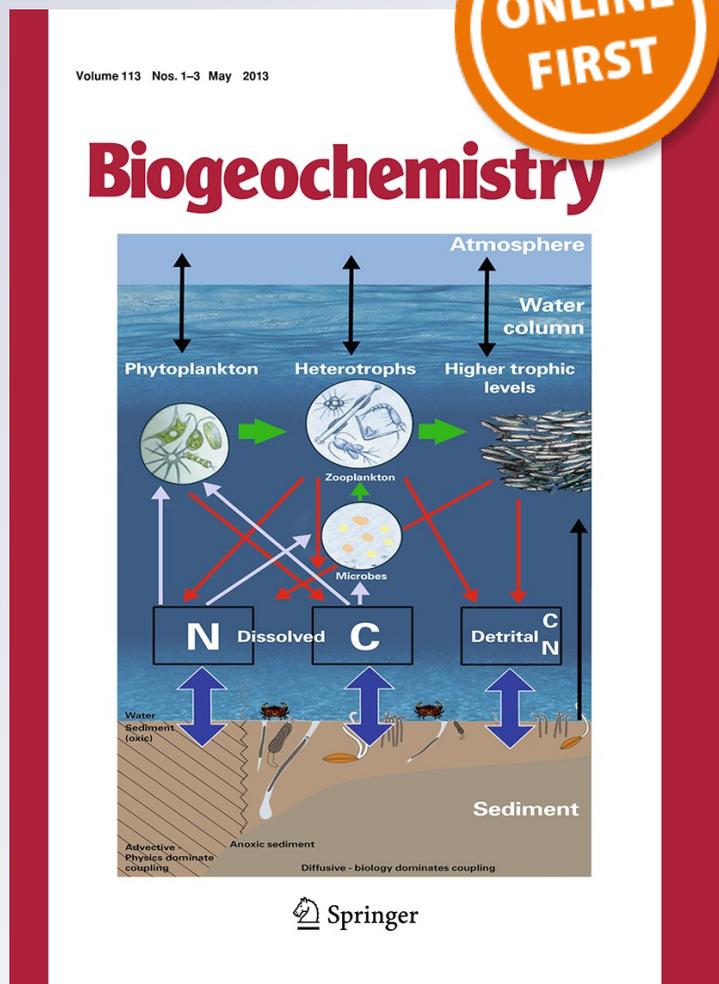
# *Substrate limitation of sediment methane flux, methane oxidation and use of stable isotopes for assessing methanogenesis pathways in a small arctic lake*

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# Substrate limitation of sediment methane flux, methane oxidation and use of stable isotopes for assessing methanogenesis pathways in a small arctic lake

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**Abstract** Among predicted impacts of climate change in the Arctic are greater thaw depth and shifts in vegetation patterns and hydrology that are likely to increase organic carbon and nutrient loading to lakes. We measured substrate limitation of sediment methane (CH<sub>4</sub>) flux, examined pathways of methanogenesis, and potential CH<sub>4</sub> oxidation using stable isotope labeled acetate in intact sediment cores from arctic lake GTH 112 (68°40'20"N, 149°14'57"W). We hypothesized that the acetoclastic pathway would dominate methanogenesis, reflecting dissolved organic carbon supply from the surrounding landscape, and that sediment CH<sub>4</sub> flux would be stimulated by addition of acetate. Experiments demonstrated acetate limitation of sediment CH<sub>4</sub> flux with short-

term CH<sub>4</sub> flux response to availability of acetate, high rates of CH<sub>4</sub> oxidation, and strong dominance of the acetoclastic over the hydrogenotrophic methanogenic pathway. The experiments also indicated that isotopic fractionation effects during isotope enrichment experiments are large during methanogenesis and can alter the methanogenic pathways being investigated. Under oxic conditions, CH<sub>4</sub> oxidation at the sediment–water interface or in the water column is likely to account for much of diffusive CH<sub>4</sub> flux, but under anoxic hypolimnetic conditions and increased substrate availability, conditions that are likely to occur with climate change, sediment CH<sub>4</sub> flux will likely increase, with oxidation utilizing a smaller portion of sediment CH<sub>4</sub> production.

**Keywords** Sediment CH<sub>4</sub> flux · CH<sub>4</sub> oxidation · Methanogenic pathways · Acetate limitation · Isotopic fractionation effects · Arctic lake

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## Introduction

Methanogenesis is well known to occur in freshwater sediments where it serves as the primary route for anaerobic decomposition of organic matter (Rudd and Hamilton 1978; Sinke et al. 1992; Nusslein et al. 2003; Maerki et al. 2009), although other pathways for anaerobic decomposition also occur and may be important under some conditions (e.g., Wetzel 2001). Atmospheric CH<sub>4</sub> concentration is increasing,

and the potential for increased atmospheric contributions due to warming in high latitude ecosystems is of concern (Whalen and Reeburgh 1992; Martens et al. 1992; Michmerhuizen et al. 1996; Walter et al. 2006). Much of the methane ( $\text{CH}_4$ ) produced in anaerobic sediments is oxidized by methane-oxidizing bacteria (MOB) at the sediment–water interface or in the water column (Hanson and Hanson 1996; Bastviken et al. 2004, 2008; Maerki et al. 2009). However, the pathways of  $\text{CH}_4$  production and the quantitative importance of  $\text{CH}_4$  oxidation are not well understood relative to most decomposition processes in aquatic ecosystems. Furthermore, arctic warming is increasing terrestrial plant production and permafrost thawing, which may alter export of organic matter and nutrients to aquatic ecosystems (Hobbie et al. 2000; Mack et al. 2004; Prowse et al. 2006; Schindler and Smol 2006; Striegl et al. 2007; Bowden 2010), thereby increasing substrate availability for both aerobic and anaerobic decomposition processes, including methanogenesis.

The two primary pathways of biogenic methanogenesis are acetoclastic and hydrogenotrophic methanogenesis (see Whalen 2005). Acetoclastic methanogens metabolize acetate to  $\text{CH}_4$  and  $\text{CO}_2$ ;  $\text{CH}_4$  is derived preferentially from the methyl carbon (Winfrey and Zeikus 1979; Krzycki et al. 1982). Hydrogenotrophic methanogenesis is a chemoautotrophic process which utilizes  $\text{CO}_2$  and  $\text{H}_2$  to produce  $\text{CH}_4$  and  $\text{H}_2\text{O}$ . Most reports show that acetoclastic methanogenesis is favored over hydrogenotrophic methanogenesis at a 2:1 ratio (Nusslein and Conrad 2000), although the proportional role of the latter increases with temperature (Glissmann et al. 2004). Eller et al. (2005) reported dominance of hydrogenotrophic  $\text{CH}_4$  production in a dimictic eutrophic lake and dominance of acetoclastic  $\text{CH}_4$  production in a eutrophic polymictic lake. However, the relative importance of the two pathways in intact lake sediments has rarely been measured.

Stable isotopes provide a potentially valuable tool for studying  $\text{CH}_4$  dynamics in ecosystems and have been important for elucidating  $\text{CH}_4$  pathways in chemostats. Stable isotopes of carbon are frequently used in ecological studies to trace carbon flow. Detritus and consumers typically have similar  $\delta^{13}\text{C}$  values as their parent photosynthetic source material and food source, respectively, because preference for the lighter isotope ( $^{12}\text{C}$ ) during processing, or fractionation, is usually relatively small (typically  $\leq 1$  ‰;

Peterson and Fry 1987). However, fractionation during methanogenesis is typically relatively large, resulting in  $\delta^{13}\text{C}\text{-CH}_4$  values that are considerably more negative than that of either the precursor organic compound or  $\text{CO}_2$  (e.g., Whiticar et al. 1986; Conrad 2005). As a consequence of fractionation effects, fermentation of acetate commonly results in  $\delta^{13}\text{C}\text{-CH}_4 = -65$  to  $-50$  ‰ relative to organic matter (e.g., C3 plants;  $\delta^{13}\text{C} = -26$  to  $-28$  ‰) and  $\text{CO}_2$  reduction commonly results in  $\delta^{13}\text{C}\text{-CH}_4 = -110$  to  $-60$  (Whiticar and Faber 1986). As with all reactions involving isotopes,  $\delta^{13}\text{C}\text{-CH}_4$  values derived from both pathways will also reflect reaction conditions (Peterson and Fry 1987). Furthermore, fractionation of  $\text{CH}_4$  during oxidation is also variable but usually large, depending on the methanotroph type (Eller et al. 2005), as well as reaction conditions (e. g., Whiticar and Faber 1986), often reported to be on the order of  $-20$  ‰ (Jones and Grey 2011). In situ measures of  $\delta^{13}\text{C}\text{-CH}_4$  alone do not necessarily provide much pathway information because such measures reflect net outcome of methanogenesis,  $\text{CH}_4$  oxidation, as well as environmental conditions that affect fractionation (Conrad 2005).

In the Arctic Foothills Region of Alaska, near the Toolik Lake Research Station, there is a high density of relatively small lakes with high landscape connectivity (e.g., Hershey et al. 2006a). In these lakes, both benthic and pelagic primary productivity are very low (Whalen et al. 2006, 2008), while benthic secondary production is relatively high (Northington et al. 2010). Some lakes are brown-stained, reflecting high inputs of terrestrially-derived dissolved organic carbon (DOC) (e.g., Whalen and Cornwell 1985; Peterson et al. 1986). Smaller lakes in the region mix intermittently in the summer, often experiencing periods of low hypolimnetic dissolved oxygen, and have dominant benthic food web components that utilize methane-derived carbon (Hershey et al. 2006b, 2010; Gentzel et al. 2012; Medvedeff and Hershey 2012). Our previous studies in Lake GTH 112 ( $68^\circ 40' 20''\text{N}$ ,  $149^\circ 14' 57''\text{W}$ ), a small arctic lake in the region, have shown well developed communities of methanogenic and  $\text{CH}_4$  oxidizing bacteria in surficial sediments (Gentzel et al. 2012). To better understand constraints on sediment  $\text{CH}_4$  flux in Lake GTH 112, we conducted experiments to measure the importance of acetate limitation and  $\text{CH}_4$  oxidation to sediment  $\text{CH}_4$  flux and the pathways of methanogenesis. We hypothesized

that acetoclastic methanogenesis would be the dominant methanogenic pathway, reflecting supply of DOC from the landscape, and that this pathway would be stimulated by substrate addition.

## Methods

### Site description

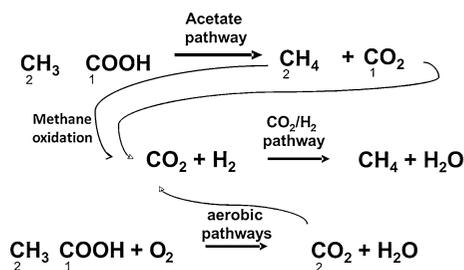
Lake GTH 112 is located in the vicinity of the Toolik Lake Arctic Research Station in arctic Alaska. It is located in rolling tundra on an ice-rich silt deposit dating to the Pleistocene (Hamilton 2002). The lake has no inlet, a low-gradient outlet, a maximum depth of ~5.6 m, a mean depth of 2.2 m, a surface area of 2.8 ha, and with soft sediments throughout the basin. It is ice covered beginning in September through early June. When the lake was sampled through ice cover on 4 May 2009, bottom water temperature was 1.5 °C and dissolved oxygen (DO) was 0.4 mg l<sup>-1</sup>. The lake is polymictic in some years, but it remains stratified from late June through early to mid-August in other years; it was stratified during the period when experiments were conducted (July of 2009 and 2010). Hypolimnetic temperature ranged from 7.1 to 8.1 °C, and DO ranged from 0.8 to 1.7 mg l<sup>-1</sup>. Measurements of CH<sub>4</sub> in surface waters during the study period (0.54 ± 0.07 μM; mean ± SE; n = 5) show supersaturation relative to the atmosphere.

### <sup>13</sup>C-acetate enrichment experiments to trace methanogenic pathways

We conducted experiments in 2009 and 2010 to determine whether methanogenesis was limited by substrate availability and evaluate the dominant pathway of sediment methanogenesis. Pathways were studied by measuring CH<sub>4</sub> flux and δ<sup>13</sup>C-CH<sub>4</sub> from intact sediment cores from Lake GTH 112 amended with <sup>13</sup>C-acetate labeled as either 1-acetate (carboxyl-labeled) or 2-acetate (methyl-labeled), or enriched with unlabeled acetate (2010 only), and by using natural abundance of δ<sup>13</sup>C-CH<sub>4</sub> and δ<sup>13</sup>C-CO<sub>2</sub> (Whiticar et al. 1986). In 2010, flux of CO<sub>2</sub> and δ<sup>13</sup>C-CO<sub>2</sub> was also measured. 1-acetate and 2-acetate treatments were selected to provide insight into the relative importance of acetoclastic and hydrogenotrophic methanogenesis

(Fig. 1). During aerobic metabolism (which uses both C groups), methyl-C is preferentially respired to CO<sub>2</sub> (Winfrey and Zeikus 1979). Depending on conditions, however, some of the carboxyl group may also be respired to CO<sub>2</sub> with the remainder retained as bacterial biomass. Acetoclastic methanogens utilize the methyl group on acetate to produce CH<sub>4</sub> (Zinder et al. 1984). Thus, 2-acetate utilized by acetoclasts would be detected as <sup>13</sup>C-CH<sub>4</sub>, while 2-acetate utilized by aerobes and other anaerobes would be primarily detected as <sup>13</sup>C-CO<sub>2</sub>. <sup>13</sup>C-CO<sub>2</sub> produced from 2-acetate could be utilized by hydrogenotrophs to produce <sup>13</sup>C-CH<sub>4</sub>, which would either be oxidized by MOB or recovered as <sup>13</sup>C-CH<sub>4</sub> in the overlying water (Fig. 1). Thus, <sup>13</sup>C-CH<sub>4</sub> detected from 2-acetate treatments would have been produced directly through acetoclastic methanogenesis, and indirectly through hydrogenotrophic methanogenesis. <sup>13</sup>C-CH<sub>4</sub> detected from 1-acetate would have been derived through hydrogenotrophic methanogenesis. Furthermore, <sup>13</sup>C-CO<sub>2</sub> produced from any source could be converted back to acetate by homoacetogens, a process that was favored over hydrogenotrophic methanogenesis in profundal sediments of Lake Constance (Schulz and Conrad 1996).

### Aerobic acetate metabolism and methanogenic pathways



**Fig. 1** Pathways for methanogenesis and aerobic metabolism of acetate illustrating the dominant expected incorporation of <sup>13</sup>C into <sup>13</sup>C-CH<sub>4</sub> and <sup>13</sup>C-CO<sub>2</sub> from 1-acetate and 2-acetate into gas products. Designation of acetate carbon atoms is indicated below each atom. Reactions are not shown in balance because some carbon is incorporated into microbial biomass; complete metabolism of acetate incorporates both carbon isotopes into gas products. Balanced reactions are as follows. Aerobic metabolism CH<sub>3</sub>-COOH + 2O<sub>2</sub> → 2CO<sub>2</sub> + 2H<sub>2</sub>O. Acetoclastic methanogenesis CH<sub>3</sub>-COOH → CO<sub>2</sub> + CH<sub>4</sub>. Hydrogenotrophic methanogenesis CO<sub>2</sub> + 4H<sub>2</sub> → CH<sub>4</sub> + 2H<sub>2</sub>O. See text for discussion

In both years, experiments were conducted using intact sediment cores collected with a KB corer (4.5 cm internal diameter, ID) from ~5.5 m in Lake GTH 112 in early July. Cores were returned to the Toolik Lake field station and extruded into 25 cm long, 4.5 cm ID incubation cores to achieve a sediment core height of ~12 cm, and sealed at the bottom with acrylic bottoms with o-ring seals. In 2009, overlying water in cores was adjusted to 0.2 and 2 mM acetate concentration treatments (low acetate and high acetate, respectively; Table 1). The High acetate treatment was designed to be low enough to avoid anoxia based on an earlier experiment in cores from the same lake using 3 mM acetate concentration (Medvedeff and Hershey 2012). Experimental cores were incubated with acetate amended Lake GTH 112 hypolimnetic water for 4 weeks, and treatment water was removed and replaced weekly. During this time, cores were uncapped, anchored in water baths containing recirculated water from the epilimnion of Toolik Lake and covered with two layers of shade cloth. This design was intended to provide time for labeled acetate products to reach equilibrium in the surficial sediments. However, since consumption of acetate would reduce concentration from initial levels and labeled gases could escape to the atmosphere, there was no expectation that all label would be recovered or that acetate could be accounted for through mass balance.

Prior to measurement of CH<sub>4</sub> flux, cores were transferred to an incubation facility at the Toolik Lake field station. Overlying water was removed and replaced with Lake GTH 112 hypolimnetic water leaving no gas headspace, stirbars were added and cores were capped with acrylic tops which had gas-tight 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 (modified from Gettel et al. 2007). This apparatus was designed to prevent establishment of chemical stratification and to maintain a gas–water equilibrium within the cores while not disturbing the sediment–water interface. Cores were incubated at 8 °C in the dark and sampled initially, at 12 h, and at 24 h for measurements of CH<sub>4</sub> and δ<sup>13</sup>CH<sub>4</sub> (24 h only) by removing 3-ml samples with a syringe from approximately mid-depth of the overlying water, and injecting the sample into a pre-evacuated, N<sub>2</sub> filled exetainer that had been acidified with 0.1 ml of 1 N HCl. Sample water was replaced with hypolimnetic water from Lake GTH 112. Following the 24-h sampling event, the overlying water was removed and replaced with hypolimnetic water that had been bubbled with N<sub>2</sub> to achieve anoxia. The cores were incubated as above for an additional 24 h, with sampling as described above.

Samples were analyzed for CH<sub>4</sub> concentration using flame-ionization detection gas chromatography (FID-GC; Shimadzu GC-8A instruments). Operating conditions for FID-GC analysis: 1/8" diameter × 1 m length molecular sieve 5A (60/80) column; column temperature = 90 °C; injector and detector temperatures = 140 °C; carrier gas = ultra-high purity N<sub>2</sub> at 33 ml min<sup>-1</sup> flow rate. Precision of analysis was <1 % at 10 ppm CH<sub>4</sub> (McGowan 2012). Time course CH<sub>4</sub> concentrations were examined for each core to confirm that CH<sub>4</sub> accumulation was approximately linear by visual inspection, and CH<sub>4</sub> flux from the sediments was determined over 24 h as the rate of change in CH<sub>4</sub> concentration between t<sub>24</sub> and t<sub>0</sub>, correcting for sediment surface area, volume of overlying water, and equilibration of the gas into the headspace of the exetainers (Whalen and Reeburgh

**Table 1** Experimental conditions for acetate amendment experiments conducted in 2009–2010 in cores (~5.5 m = Z<sub>max</sub>) from Lake GTH 112

Years	Concentrations (mmol)	Acetate treatments	Anoxic treatment	Replication	Incubation time with acetate amendment (week prior to flux exp.)	Incubation temp. prior to flux exp. (°C)	Flux exp. temp. (°C)
2009	0.2 2	Control, 1-acetate, 2-acetate	Yes	6	4	~15	8
2010	0.1	Control, unenriched acetate, 1-acetate, 2-acetate	No	7	1	8	8

All cores were open to the atmosphere during the incubation time indicated, then capped for measurement of CH<sub>4</sub> flux over 24 h

1992).  $\delta^{13}\text{C}\text{-CH}_4$  samples from  $t_{24}$  were sent to the UC Davis stable isotope facility for analyses.

In the 2010 experiment, acetate amendments (1-acetate, 2-acetate, and unlabeled acetate) were designed to be at a low level (0.1 mM) to minimize altering substrate availability for methanogens compared to natural conditions, and also to avoid the potential problem of anoxia developing after cores were capped. An unlabeled acetate treatment was included in order to better evaluate the importance of isotopic fractionation to sediment  $\text{CH}_4$  flux during the experiment. Although not measured in our study, Bretz (2012) measured acetate concentration of 0.1 mM in sediments from 5 to 6 m in Toolik Lake and Lake NE-14, both of which are less productive than Lake GTH 112 (Whalen et al. 2008). In the 2010 experiment, cores were incubated at 8 °C in the dark with substrate amendments for 1 week with no refreshment of treatments. Accordingly, 0.1 mM enrichment in the 2010 experiment was likely <a twofold increase in ambient acetate concentration initially, which would have declined during the incubation prior to flux measurements. Following the incubation period, overlying core water was replaced with hypolimnetic water from Lake GTH 112, and  $\text{CH}_4$  flux and  $\delta^{13}\text{C}\text{-CH}_4$  were measured as described above for 2009. We also measured  $\text{CO}_2$  flux and  $\delta^{13}\text{C}\text{-CO}_2$  based on samples taken at the same time points and in the same manner as those for  $\text{CH}_4$  flux and  $\delta^{13}\text{C}\text{-CH}_4$ .  $\text{CO}_2$  concentration was measured using thermal conductivity detection gas chromatography (TCD-GC). Operating conditions for TCD-GC analysis: 1/8"  $\times$  2 m porapak N column (80/100); column temperature = 40 °C; injector detector temperatures = 140 °C, current = 140 mA; carrier gas = ultra-high purity He at 30 ml min<sup>-1</sup> flow rate. Precision of analysis was <1 % at 351 ppm  $\text{CO}_2$  (Whalen and Reeburgh 2000). No methane oxidation inhibition treatments were conducted; this reflected the logistic constraint of availability of incubator space.  $\delta^{13}\text{C}\text{-CO}_2$  samples were also sent to the UC Davis Stable Isotope Facility for analyses.

#### Data analysis

All data sets were examined for homogeneity of variance and natural log (ln) transformed as needed to satisfy the homogeneity assumption. For data sets requiring transformation that contained negative values, the smallest integer needed to result in all positive

values for that data set was added to all observations prior to ln transformation. The 2009 acetate enrichment experiment was analyzed with a two-way ANOVA with interaction. Significant main effects and interactions were followed by one-way ANOVAs and Tukey's pairwise comparisons or *t* tests for three and two treatment groups, respectively. The 2010 acetate enrichment experiment was analyzed with one-way ANOVAs followed by Tukey's pairwise comparisons.

## Results

### 2009 labeled acetate experiment

Sediment  $\text{CH}_4$  flux varied significantly among acetate treatments in 2009 (Table 2). Overall, flux was higher under anoxic compared to ambient conditions, although responses to  $\text{O}_2$  condition were different among acetate treatments (Fig. 2a; Table 2). Only the high 1-acetate and high 2-acetate treatments showed positive sediment  $\text{CH}_4$  flux, regardless of  $\text{O}_2$  condition. Under ambient conditions, the only significant differences were between the high 1-acetate treatment and the control and low 2-acetate treatments. Under anoxic conditions, sediment  $\text{CH}_4$  flux was significantly greater in the high 2-acetate treatment compared to all other treatments. None of the other treatment comparisons were significant. Anoxic conditions resulted in significantly greater sediment  $\text{CH}_4$  flux than ambient  $\text{O}_2$  conditions in control and low 2-acetate treatments only (Fig. 2a; Table 2).

$\delta^{13}\text{C}\text{-CH}_4$  was significantly affected by both acetate treatment and  $\text{O}_2$  condition. There was no significant interaction effect (Fig. 2b; Table 2). Greatest label incorporation into  $\text{CH}_4$  occurred in the high 2-acetate treatment, which was approximately sixfold more enriched than the high 1-acetate treatment. The high 1-acetate treatment had significantly greater  $\delta^{13}\text{C}\text{-CH}_4$  compared to the low 1-acetate, low 2-acetate and control treatments, but  $\delta^{13}\text{C}\text{-CH}_4$  in the two low acetate treatments were only slightly enriched above the control treatment.  $\delta^{13}\text{C}\text{-CH}_4$  was significantly lower under anoxic compared to ambient  $\text{O}_2$  conditions across all treatments, although the magnitude of the differences was small. This difference likely reflected isotopic fractionation of carbon isotopes during  $\text{CH}_4$  oxidation.

**Table 2** Summary of ANOVA analyses for sediment CH<sub>4</sub> and CO<sub>2</sub> (2010 only) flux,  $\delta^{13}\text{C-CH}_4$  and  $\delta^{13}\text{C-CO}_2$  from acetate enrichment experiments in 2009 and 2010. Treatments not

shown in pairwise contrasts were not significantly different from any other treatments

Analysis	F-ratio or t	n	df	P<	Significant pairwise contrasts
2009 CH <sub>4</sub> 2-way model	6.71	57	9,47	0.0001	
Acetate effect	10.51	57	4,47	0.0001	
O <sub>2</sub> condition effect	6.94	57	1,47	0.01	Anoxic > ambient
Acetate × O <sub>2</sub> condition interaction	3.27	57	4,47	0.02	
2009 CH <sub>4</sub> 1-way ANOVAs/ <i>t</i> test					
Acetate ambient condition	4.07	28	4,23	0.01	High 1-acetate > low 2-acetate = control
Acetate anoxic condition	18.16	29	4,24	0.0001	High 2-acetate > high 1-acetate = low 1-acetate = low 2-acetate = control
O <sub>2</sub> effect control	9.14	12	10	0.0001	Anoxic > ambient
O <sub>2</sub> effect high 1-acetate	0.96	12	10	0.4	n. s.
O <sub>2</sub> effect high 2-acetate	1.67	12	10	0.1	n. s.
O <sub>2</sub> effect low 1-acetate	1.07	12	10	0.3	n. s.
O <sub>2</sub> effect low 2-acetate	5.05	11	9	0.0007	Anoxic > ambient
2009 $\delta^{13}\text{C-CH}_4$ 2-way model	223.25	58	9,48	0.0001	
Acetate effect	499.31	58	4,48	0.0001	High 2-acetate > high 1-acetate > low 2-acetate = low 1-acetate = control
O <sub>2</sub> condition effect	8.95	58	1,48	0.004	Ambient > anoxic
Acetate × O <sub>2</sub> condition interaction	0.93	58	4,48	0.5	n. s.
2010 CH <sub>4</sub> 1-way ANOVA	5.25	28	3,24	0.006	1-acetate > unlabeled acetate = control
2010 $\delta^{13}\text{C-CH}_4$ 1-way ANOVA	56.19	28	3,24	0.0001	2-acetate > 1-acetate > unlabeled acetate = control
2010 CO <sub>2</sub> 1-way ANOVA	0.52	28	3,24	0.67	n. s.
2010 $\delta^{13}\text{C-CO}_2$ 1-way ANOVA	157.32	26	3,22	0.0001	2-acetate > 1-acetate > unlabeled acetate = control

### 2010 labeled acetate experiment

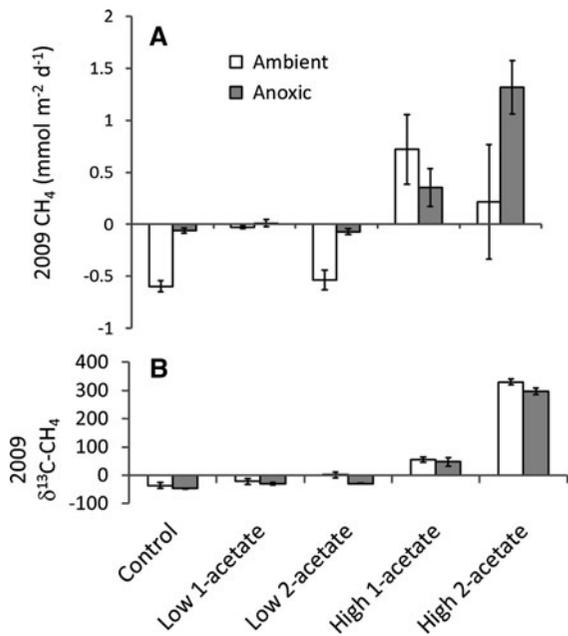
In the 2010 acetate enrichment experiment, control and unlabeled acetate treatments showed no significant net sediment CH<sub>4</sub> flux. Greatest CH<sub>4</sub> flux occurred in the 1-acetate treatment, which differed significantly from CH<sub>4</sub> flux in the unlabeled acetate and control treatments. Sediment CH<sub>4</sub> flux was also positive in the 2-acetate treatment, but it did not differ significantly from any other treatment (Fig. 3a; Table 2).

$\delta^{13}\text{C-CH}_4$  in the 2-acetate treatment was ~11-fold greater than the 1-acetate treatment.  $\delta^{13}\text{C-CH}_4$  was also significantly greater in the 1-acetate treatment than in either the control or unlabeled acetate treatment

(Fig. 3b; Table 2), reflecting the lack of isotope enrichment in either of the latter treatments.

CO<sub>2</sub> flux did not differ significantly among treatments, although the control treatment appeared to show slightly lower flux rates, and some cores showed slightly negative CO<sub>2</sub> flux compared to the acetate treatments (Fig. 3c; Table 2).

Both 1-acetate and 2-acetate treatments were highly enriched in <sup>13</sup>C-CO<sub>2</sub> and significantly more enriched than control and unlabeled acetate treatments. 2-acetate also showed significantly greater <sup>13</sup>C-CO<sub>2</sub> enrichment than 1-acetate (Fig. 3d; Table 2). Control and Unlabeled acetate treatments had  $\delta^{13}\text{C-CO}_2$  values that were consistent with expected background values (−13 and −14 ‰, respectively).



**Fig. 2** Labeled acetate experiment conducted in 2009. **a** Mean  $\pm$  SE CH<sub>4</sub> flux (mmol m<sup>-2</sup> day<sup>-1</sup>); and **b** mean  $\pm$  SE δ<sup>13</sup>C-CH<sub>4</sub>. See Table 2 for statistical comparisons

**Discussion**

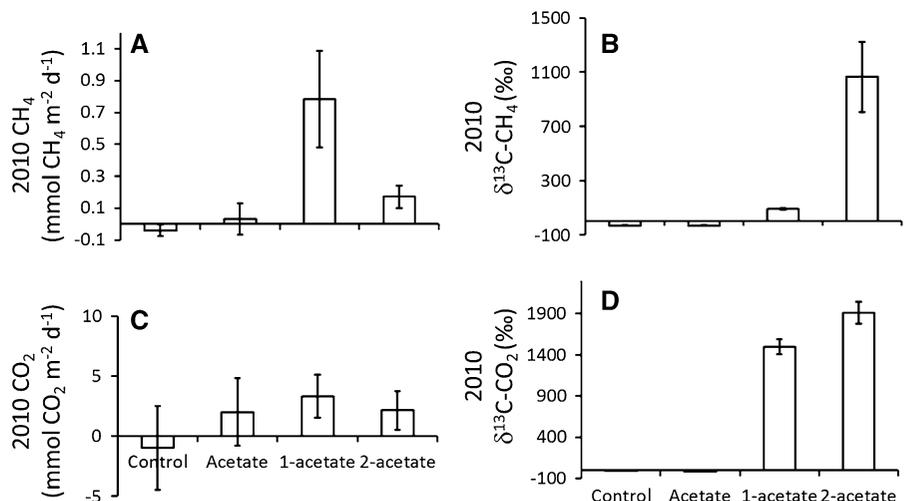
2009 labeled acetate incubation experiment

Examination of CH<sub>4</sub> flux from sediments under ambient compared to anoxic treatments in the 2009 acetate experiment demonstrated that CH<sub>4</sub> oxidation at the sediment–water interface is variable but can be a considerable CH<sub>4</sub> sink. Negative CH<sub>4</sub> flux in ambient

control cores resulted from oxidation of CH<sub>4</sub> present in the overlying water during the experiment which was supplied from replenishment of core water with Lake GTH 112 hypolimnetic water prior to capping of cores as well as from oxidation of CH<sub>4</sub> produced in the cores while they were capped. Comparison of CH<sub>4</sub> flux in ambient and anoxic control cores illustrates that the sediment–water interface can serve as a CH<sub>4</sub> sink for water column CH<sub>4</sub> when rate of methanogenesis is relatively low. Such a condition might occur, for example, if a lake is not stratified and inflowing stream water is supersaturated in CH<sub>4</sub>. In high 2-acetate cores, where substrate limitation was mitigated (discussed below), positive CH<sub>4</sub> flux under ambient conditions compared to increased CH<sub>4</sub> flux under anoxia illustrates a high CH<sub>4</sub> oxidation potential at the sediment–water interface and in the bottom water when CH<sub>4</sub> supply from the sediments is high. High rates of CH<sub>4</sub> oxidation at the sediment–water interface are consistent with observed high concentrations of CH<sub>4</sub> oxidizing bacteria in bottom water and 1–2 mm into the sediment profile in Lake GTH 112 (Gentzel et al. 2012). Large scale comparisons have shown that much of the CH<sub>4</sub> produced in lake sediments is oxidized in the water column (Bastviken et al. 2004, 2008). Our results show that in a lake with hypoxic bottom water, not only does the sediment–water interface scavenge much of the CH<sub>4</sub> as it diffuses upward, but it also may serve to oxidize CH<sub>4</sub> from the water column.

The overall increase in CH<sub>4</sub> flux in acetate-enriched treatments compared to controls demonstrate substrate limitation of methanogenesis, but pairwise comparisons

**Fig. 3** Labeled acetate experiment conducted in 2010. **a** Mean  $\pm$  SE CH<sub>4</sub> flux (mmol m<sup>-2</sup> day<sup>-1</sup>), **b** mean  $\pm$  SE δ<sup>13</sup>C-CH<sub>4</sub>, **c** mean  $\pm$  SE CO<sub>2</sub> flux (mmol m<sup>-2</sup> day<sup>-1</sup>), and **d** mean  $\pm$  SE δ<sup>13</sup>C-CO<sub>2</sub>. See Table 2 for statistical comparisons



showed that added acetate mitigated substrate limitation only in high acetate treatments, depending on  $^{13}\text{C}$  label position and  $\text{O}_2$  condition. Lack of a significant response to the low acetate treatments was not due to  $\text{CH}_4$  oxidation because anoxic cores also did not exhibit significant positive  $\text{CH}_4$  flux. This lack of a response suggests that the added acetate was largely or entirely consumed prior to capping of cores, resulting in substrate limitation at the time flux measurements were made. This conclusion is further supported by the result that  $\delta^{13}\text{C}\text{-CH}_4$  showed little or no enrichment over controls in the low acetate treatments. Either most or all of the  $\text{CH}_4$  captured for measurement of  $\delta^{13}\text{C}\text{-CH}_4$  in the low acetate treatments was derived from the overlying water that had been replaced with Lake GTH 112 hypolimnetic water prior to capping the cores, or the  $\text{CH}_4$  was produced in these treatments from deeper unenriched substrates.  $\text{CH}_4$  flux from sediment cores collected from the same depth in Lake GTH 112 in 2009 that were capped immediately after collection showed that  $\text{CH}_4$  flux from sediments was  $0.9 \text{ mmol m}^{-2} \text{ day}^{-1}$  (Gentzel et al. 2012), which was within range of that observed in the High acetate cores that had been incubated for 1 month (Fig. 2a). Thus, sediment  $\text{CH}_4$  flux in Lake GTH 112 was substrate limited and appeared to be responding to relatively short term availability of substrate.

Overall greater  $\text{CH}_4$  flux under anoxia demonstrates that the sediment–water interface and bottom water are important sites of  $\text{CH}_4$  oxidation, regulating  $\text{CH}_4$  flux to the water-column. Increased  $\text{CH}_4$  flux has been observed commonly under anoxia in lake sediments (e.g., Huttunen et al. 2006; Gentzel et al. 2012). The high variability in the ambient high 2-acetate treatment suggests that  $\text{O}_2$  conditions, which we did not measure, probably were not consistent across replicate cores. Cores with lower  $\text{CH}_4$  flux may have remained aerobic while those with high  $\text{CH}_4$  flux may have become anoxic while they were capped. Greater  $\text{CH}_4$  flux under anoxia in the high 2-acetate compared to the high 1-acetate treatment is most likely due to isotopic fractionation during acetate assimilation by acetoclasts. The carboxyl end is the reactive site on the acetate molecule for microbial uptake (Ferry 1992), such that the lighter ( $^{12}\text{C}$ ) carboxyl site in 2-acetate would be more readily assimilated by acetoclasts than the heavier ( $^{13}\text{C}$ ) carboxyl site in 1-acetate, leading to greater  $\text{CH}_4$  flux from 2-acetate compared to 1-acetate under anoxia. Under ambient conditions, the result

was qualitatively different. The pattern of greatest  $\text{CH}_4$  flux in the high 1-acetate treatment compared with intermediate flux from high 2-acetate illustrates that the unlabeled carboxyl site of 2-acetate was also favored by aerobes. Thus, there was relatively greater availability of 1-acetate for assimilation by methanogens, resulting in the observed pattern of lower  $\text{CH}_4$  production from 2-acetate compared to 1-acetate under ambient conditions, i.e., aerobes effectively outcompeted methanogens for 2-acetate under those conditions, while discriminating against 1-acetate. Presumably, if the incubation period had been longer prior to capping of cores, the substrate depletion that occurred in low acetate treatments would also have occurred in the high acetate treatments. Note that these processes would have occurred during the incubation period as well as during the flux study, such that substrate availability, which we did not measure, may have been different during flux measurements between the two high acetate treatments. However, the significantly greater  $\text{CH}_4$  production in anoxic high 2-acetate cores compared to all other treatments illustrates that substrate depletion did not occur.

$\delta^{13}\text{C}\text{-CH}_4$  data from the 2009 high acetate treatments illustrate dominance of the acetoclastic methanogenic pathway. Fourfold greater  $\delta^{13}\text{C}\text{-CH}_4$  enrichment in the anoxic high 2-acetate treatment compared to the high 1-acetate treatment shows a greater importance of the acetoclastic over the hydrogenotrophic pathway because  $^{13}\text{C}\text{-CH}_4$  from 2-acetate would be incorporated from both pathways while hydrogenotrophs would be expected to produce  $^{13}\text{C}\text{-CH}_4$  only indirectly from 1-acetate (Fig. 1). However, quantifying the relative importance of the two pathways is precluded because the availability of  $^{13}\text{C}\text{-CO}_2$  to support hydrogenotrophic production of  $^{13}\text{C}\text{-CH}_4$  was unknown in 2009. Furthermore, availability of  $^{13}\text{C}\text{-CO}_2$  may have differed between high acetate treatments due to isotopic fractionation effects during both aerobic and methanogenic metabolic processes that, as described above, appeared to have been large and were operating on an acetate amendment that was only 7.2 % enriched in  $^{13}\text{C}$  of the target atom. However, the observed  $\delta^{13}\text{C}\text{-CH}_4$  value of  $-46.5 \text{ ‰}$  in the anoxic control cores also indicates strong dominance of the acetoclastic pathway;  $^{13}\text{C}\text{-CH}_4$  produced through hydrogenotrophy is typically more depleted, with values as low as  $-110 \text{ ‰}$  (Whiticar and Faber 1986).

## 2010 labeled acetate incubation experiment

Some control cores in the 2010 experiment appeared to exhibit negative CO<sub>2</sub> flux, although overall CO<sub>2</sub> flux across replicate control cores was not significantly different from zero. Because the flux experiment occurred in the dark, mechanisms for negative CO<sub>2</sub> flux include hydrogenotrophy as well as various other microbial CO<sub>2</sub> consuming processes that we did not evaluate in our experiments (e.g., homoacetogenesis, nitrification, metal oxidation, sulfur oxidation).

Sediment CH<sub>4</sub> flux in the 2010 acetate experiment also suggests substrate limitation, and provides additional insight into the importance of fractionation effects in stable isotope studies of methanogenesis. Lack of significant stimulation of sediment CH<sub>4</sub> flux compared to controls in the unlabeled acetate treatment indicates that the 0.1 mM acetate amendment, followed by a 1-week incubation, reasonably approximated a low level amendment above background acetate concentration. The significant increase in sediment CH<sub>4</sub> flux in the 1-acetate treatment over both control and unlabeled acetate treatments is similar to the pattern observed in the ambient high acetate treatments in 2009, where high 1-acetate showed the greatest CH<sub>4</sub> flux response. Substrate depletion did not occur in the labeled acetate treatments in 2010 as it did in low acetate treatments in 2009 even though initial acetate concentration was twofold higher in 2009 than in 2010 (Table 1). These different responses reflect differences in incubation temperature prior to CH<sub>4</sub> flux measurements; temperature was about 15 °C in 2009, compared to 8 °C in 2010. Significantly greater CH<sub>4</sub> flux in the 1-acetate and intermediate CH<sub>4</sub> flux in the 2-acetate treatment compared to the unlabeled acetate treatment clearly illustrates that fractionation effects were large overall but greater for 1-acetate. Furthermore, intermediate CH<sub>4</sub> flux and greater δ<sup>13</sup>C-CO<sub>2</sub> from 2-acetate compared to 1-acetate indicates that 2-acetate was preferentially used by aerobes over 1-acetate, decreasing its availability to methanogens.

Environmental data on δ<sup>13</sup>C-CH<sub>4</sub> and its precursors can be used to estimate the relative importance of methanogenic pathways when the isotopic fractionation effect (α-value) is measured or can be estimated, but tracer experiments with carefully controlled environmental conditions have been used to provide more precise estimates (see Conrad 2005). There are many

caveats to determining fractionation because there are a large number of competing processes that affect δ<sup>13</sup>C of CH<sub>4</sub> and as well as δ<sup>13</sup>C of precursor CO<sub>2</sub> and acetate (Whiticar 1999; Conrad 2005). Furthermore, slurry experiments with inhibitors where experimental conditions can be more precisely controlled than in cores have also been used to measure pathway potential (e.g., Lofton 2012), but, unlike cores, slurries do not mimic vertical stratification in lake sediments. Our 2009 experiment illustrated that fractionation effects are large and that δ<sup>13</sup>C-CH<sub>4</sub> changes during oxidation, such that, as noted by Conrad (2005), measured δ<sup>13</sup>C-CH<sub>4</sub> in the overlying water would not have been the same as microbially produced δ<sup>13</sup>C-CH<sub>4</sub> in the sediments. This surely also would have been true in the 2010 experiment where cores were aerobic throughout the study. Reported α-values for methanogenesis range from 1.007 to 1.099, but also vary considerably with environmental conditions and label position (reviewed in Conrad 2005). A crude estimate of α for hydrogenotrophic production of CH<sub>4</sub> can be obtained from natural abundance of δ<sup>13</sup>C-CH<sub>4</sub> and δ<sup>13</sup>C-CO<sub>2</sub> as follows (Whiticar et al. 1986; Conrad 2005):

$$\alpha_c = (\delta^{13}\text{C-CO}_2 + 1000) / (\delta^{13}\text{C-CH}_4 + 1000) \quad (1)$$

where α<sub>c</sub> = apparent carbon fractionation factor by hydrogenotrophs.

In freshwater sediments, α<sub>c</sub> > 1.065 indicates dominance of hydrogenotrophy, while α<sub>c</sub> < 1.055 indicates dominance of acetatoclastic methanogenesis (Whiticar et al. 1986). Using data from 2010 control and unlabeled acetate cores (Fig. 3) in Eq. 1, α<sub>c</sub> = 1.019 and α<sub>c</sub> = 1.017, respectively, suggesting virtually complete dominance of acetoclastic methanogenesis in the absence of <sup>13</sup>C enrichment; these values fall clearly in the 1.007–1.027 range of literature reports for acetoclastic methanogenesis in pure cultures (see Conrad 2005).

Our 2010 tracer experiment does not provide a realistic tool for precise estimation of pathways in Lake GTH 112 sediments despite the low level of acetate enrichment because fractionation effects were clearly important, different between treatments, and would have changed during oxidation (as illustrated for the 2009 experiment). It does, however, illustrate the environmental potential for the two pathways as well as the impact of experimental conditions on

pathway assessment. In the 1-acetate treatment, presumably all of the  $^{13}\text{C-CH}_4$  was produced by hydrogenotrophy from  $^{13}\text{CO}_2$  (Fig. 1). If fractionation effects had been similar, the ratio of  $\delta^{13}\text{C-CH}_4:\delta^{13}\text{C-CO}_2$  in the 1-acetate treatment could be used to estimate the portion of total  $^{13}\text{C-CH}_4$  in the 2-acetate treatment that was produced by hydrogenotrophs, with the remainder of the  $^{13}\text{C-CH}_4$  produced in the 2-acetate treatment attributed to acetoclastic methanogenesis, resulting in an acetoclastic:hydrogenotrophic ratio of  $\sim 8:1$ . Higher  $\delta^{13}\text{C-CO}_2$  in the 2-acetate treatment would be expected to result in disproportionate hydrogenotrophic incorporation of  $^{13}\text{C-CO}_2$  into  $^{13}\text{C-CH}_4$ , such that an 8:1 ratio represents an upper limit for the environmental potential for hydrogenotrophy, while actual hydrogenotrophy would have been lower. An 8:1 ratio is a higher proportional acetoclastic contribution than a depth-integrated estimate of acetoclastic:hydrogenotrophic methanogenesis for nearby Lake GTH 114 (3:1) but similar to that in nearby lake E-4 (8:1) based on slurry experiments (from Lofton 2012), and higher proportional acetoclastic production than the 2:1 ratio of reported as typical for freshwater sediments (Nusslein and Conrad 2000). However, the estimate of virtually complete dominance of the acetoclastic pathway in Lake GTH 112 based on natural abundance data in control and unlabeled acetate cores may be more realistic.

## Conclusions

Substrate limitation of methanogenesis was apparent from acetate amendment experiments in both 2009 and 2010. Such short-term processing of labile organic substrates is consistent with dominance of the acetoclastic methanogenic pathway. Natural abundance of  $\delta^{13}\text{C-CH}_4$  and  $\delta^{13}\text{C-CO}_2$  indicate a virtual complete dominance of the acetoclastic pathway, but the enrichment shows that the environmental potential for hydrogenotrophy is present but considerably lower than the 2:1 ratio acetoclastic to hydrogenotrophic production that is typically reported (Nusslein and Conrad 2000). Our experiments also show that the potential for  $\text{CH}_4$  oxidation at the sediment–water interface is high. Under conditions of extreme substrate limitation of methanogenesis, sediments can serve as a net  $\text{CH}_4$  sink. Among the predicted impacts of climate change in the Arctic are shifts in vegetation

patterns, increases in thaw depth, and changes in hydrology that may increase organic carbon (see, Mack et al. 2004; Prowse et al. 2006; Striegl et al. 2007) and nutrient (Hobbie et al. 2000; Schindler and Smol 2006) loading to lakes. The observed pattern of substrate limitation suggests that  $\text{CH}_4$  flux is likely to increase if organic carbon and nutrient loading occurs. Furthermore, small arctic lakes are often hypoxic during stratification (e.g., Hershey et al. 2006b), and nutrient limited (Levine and Whalen 2001), such that increases in organic carbon and nutrient loading are likely to increase hypoxia, reducing proportional  $\text{CH}_4$  oxidation and further enhancing  $\text{CH}_4$  flux from sediments.

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