Effects of algal and terrestrial carbon on methane production rates and methanogen community structure in a temperate lake sediment

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SUMMARY

1. Sources of atmospheric CH$_4$ are both naturally occurring and anthropogenic. In fact, some anthropogenic activities may influence the production of CH$_4$ from natural sources, such as lakes.
2. Ongoing changes in the catchment of lakes, including eutrophication and increased terrestrial organic carbon export, may affect CH$_4$ production rates as well as shape methanogen abundance and community structure. Therefore, inputs from catchments to lakes should be examined for their effects on CH$_4$ production.
3. We added algal and terrestrial carbon separately to lake sediment cores and measured CH$_4$ production. We also used quantitative polymerase chain reaction and terminal restriction fragment length polymorphism to determine the effects of these carbon additions on methanogen abundance and community composition.
4. Our results indicate that CH$_4$ production rates were significantly elevated following the addition of algal biomass. Terrestrial carbon addition also appeared to increase methanogenesis rates; however, the observed increase was not statistically significant.
5. Interestingly, increased CH$_4$ production rates resulted from increases in per-cell activity rather than an increase in methanogen abundance or community compositional shifts, as indicated by our molecular analyses.
6. Overall, anthropogenic impacts on aquatic ecosystems can influence methanogenesis rates and should be considered in models of global methane cycling and climate.

Keywords: community, eutrophication, greenhouse gases, lakes, profundal

Introduction

Methane (CH$_4$), a potent greenhouse gas, continues to increase in concentration in our atmosphere. Current atmospheric CH$_4$ concentrations of 1774 ppbv are more than double pre-industrial levels of 350–800 ppbv (Loulergue et al., 2008). Freshwater ecosystems may contribute up to 103 teragrams of CH$_4$ per year, 6–16% of global CH$_4$ emissions, and are not currently included in global greenhouse gas budgets (Bastviken et al., 2004, 2011). To predict, and perhaps mitigate, future increases in atmospheric CH$_4$, we must understand factors that regulate the contribution of inland waters to the global CH$_4$ cycle and how these factors will change under future global change scenarios.

It is well accepted that lake-dwelling organisms and the processes they mediate are sensitive to anthropogenic activities in the surrounding catchment. Therefore, it is to be expected that aquatic methanogenic archaea, and methanogenesis rates, will be influenced by catchment change. Two pervasive and ongoing catchment-derived impacts on lakes are the transport of nutrients and organic matter (Schindler, 1974; Monteith et al., 2007). Critically evaluating how these important drivers of global change in aquatic ecosystems, eutrophication and ‘browning’ (Roulet & Moore, 2006) may impact lake CH$_4$ dynamics is
an important component of global climate change predictions.

Excess nutrient run-off from non-point sources into receiving aquatic systems has been an environmental concern for decades (Edmondson & Lehman, 1981; Smith, 2003; Bosch et al., 2009). Lakes as well as other aquatic systems receiving subsurface and surface run-off of the limiting nutrient phosphorus are characterised by accelerated eutrophication (Sharpley et al., 1994; Schindler et al., 2008). Subsequent sinking and decay of algal blooms creates a nutrient-rich anoxic environment suitable for fermentation and the production of acetate and H2/CO2. These organic precursors, without biogeochemical competition, can be anaerobically reduced to CH4 by methanogens (Conrad, 1999). As eutrophication, caused by anthropogenic sources, probably provides a greater supply of substrate for methanogenesis, we anticipate increased CH4 production rates within lakes under eutrophic conditions.

Environmental change not only increases nutrient concentration in lakes, it may also result in an increase in terrestrial dissolved organic carbon (DOC) in many aquatic ecosystems. In Europe and North America, freshwater ecosystems have experienced increased DOC leaching from soil (Hejzlar et al., 2003; Worrall et al., 2004; Monteith et al., 2007). Elevated export of DOC from upland soils over the past two decades is thought to have resulted from decreased soil acidity and ionic strength following policy changes for fossil fuel combustion and sulphur emissions in the 1990s (Monteith et al., 2007). Increased soil pH and lower ionic strength enhance the solubility and mobility of organic acids in soil, which allows for greater DOC export to surface waters (Driscoll et al., 2003; Evans et al., 2006). However, other studies have hypothesised that climate change (Freeman et al., 2001, 2004; Worrall, Burt & Shedden, 2003), nitrogen deposition (Findlay, 2005) and land use change (Garnett, Ineson & Stevenson, 2000) all have contributed to increased DOC export to lakes. Regardless of the reason, terrestrial organic carbon concentrations within lakes are increasing, and these terrestrial subsidies can be a source of energy for microbial populations (Bergstrom & Jansson, 2000; Kritzberg et al., 2004), perhaps including methanogens.

Observed trends in lake trophic status around the globe suggest that many lakes are changing towards a more eutrophic or humic state (Vollenweider, 1989; Smith, 2003; Monteith et al., 2007). Here, we ask whether we can expect these primarily human-driven changes to affect surface water contributions to the global CH4 cycle. To evaluate whether algal or terrestrial carbon inputs could enhance CH4 production rates, we conducted a laboratory-scale manipulation of carbon substrate supply to anoxic lake sediments. Any change in methanogenesis in response to substrate amendment would result from changes in the methanogenic archaeal community in the lake sediments. The enhanced methanogenic activity could be mediated by one or more scenarios of methanogen community change: (i) increased abundance of existing methanogens, (ii) increased per-cell activity of existing methanogens, but no change in abundance or (iii) shifts in methanogen community composition to a more efficient or active set of organisms. To assess what scenario might accompany any changes in methanogenesis activity in response to our carbon substrate supply manipulations, we used a combination of quantitative polymerase chain reaction (qPCR) and terminal restriction fragment length polymorphism (T-RFLP), targeting a gene integral to the methanogenesis biochemical pathway (mcrA).

Methods

Sampling site

Diamond Lake is an oligotrophic lake (DOC = 5.3 mg L-1, TP = 23.7 µg L-1 and pH = 8.6) located in southwest Michigan, U.S.A. (41.9°N; 85.9°W). On 29 September 2010, we collected nine replicate intact sediment cores, including overlying water, with a Kajak-Brinkhurst corer (Wildlife Supply Co., Yulee, FL, U.S.A.) at the deepest point in the lake and returned them to the laboratory. Prior to collection of our cores, we measured profiles of water temperature, dissolved oxygen and CH4 concentrations.

Core amendments, incubations and CH4 measurements

All sediment cores contained approximately 200 mL of water, 600 mL of sediment and 100 mL of headspace, which was purged with N2 gas. The nine cores were divided evenly into two treatments (algal and terrestrial) and a control. We added 7 g m-2 of dried Scenedesmus obliquus (Turpin) Kützing 1833 to the three algal cores. Each of the terrestrial cores was amended with 7 g m-2 of dried, ground maple leaves; for this purpose, freshly fallen maple leaves were collected and then dried at 60 °C. After drying, the leaves were ground to a particle size comparable to algal cells using a Thomas Wiley Mini-mill with size 60 mesh (Thomas Scientific, Swedesboro, NJ, U.S.A.) and a Wig-L-Bug grinding mill (Dentsply Rinn Corp., York, PA, U.S.A.). Although freshly fallen leaves are probably of higher quality than most terrestrial particulate carbon reaching lake sediments, we chose...
fresh leaves because they are more comparable to the algal additions in terms of quality. The three control cores were treated in a similar manner to the algal and terrestrial cores, but no additional carbon substrate was supplied. The cores, sealed with a rubber stopper fit with valved sampling ports, were incubated at about 15 °C in the dark for 2.5 weeks. Approximately every 4 days, a 5-mL sample was taken from each core’s headspace and replaced with 5 mL of N₂. The CH₄ concentration in each sample was measured with an Agilent 6890 Gas Chromatograph equipped with a flame ionising detector, using a GS carbon plot column with a length, diameter and filter size of 30 mm, 0.32 mm and 3.0 µm, respectively (Agilent Technologies, Santa Clara, CA, U.S.A.). CH₄ concentration increased linearly during the entire 2.5-week incubation in the nine sediment cores. Methanogenesis rates were inferred from the slope of linear regression fits to the concentration in each sample.

Molecular analyses

Following 2.5 weeks of incubation, overlying water and sediments were collected for DNA extractions. Filtered planktonic biomass and sediment DNA extractions were performed according to manufacturer’s protocol with a Mo-Bio DNA power soil kit (Mo Bio, Carlsbad, CA, U.S.A.). Extracted DNA served as template for quantitative PCR (qPCR) targeting the alpha subunit of methyl coenzyme reductase (mcrA). Many previous studies have used mcrA as a genetic marker to determine methanogen abundance as well as community composition (Luton et al., 2002; Earl et al., 2003; Freitag et al., 2010; Milferstadt, Youngblut & Whitaker, 2010). This gene is an excellent marker as all known methanogens possess methyl coenzyme reductase, which is responsible for the conversion of a methyl group to CH₄ (Grabarse et al., 2001).

mcrA was amplified in a 20-µL qPCR in an ep gradient S realplex² master cycler (Eppendorf, Hauppague, NY, U.S.A.), using SYBR Green as the reporter dye. Each reaction contained 1 µL of 1/100 diluted DNA sample from an incubated core, 1 × 1.5 mM MgCl₂ PCR buffer (Idaho Technology Inc.), 0.4 mM dNTPS, 1 unit of Taq, 0.2 µM mcrA-FAM-labelled (5'-GGTGGTGTGGAGATCACCACACGTTAG-3') and 0.2 µM mcrA-R (5'-TTCATTGCGCTTGGTATAG-3') primers. Each PCR was run in an ep gradient pro realplex² master cycler (Eppendorf) and contained 1 µL of 1/100 diluted DNA sample from an incubated core, 1 × 1.5 mM MgCl₂ PCR buffer (Idaho Technology Inc.), 0.4 mM dNTPS, 1 unit of Taq, 0.2 µM mcrA-FAM-labelled (5'-GGTGGTGTGGAGATCACCACACGTTAG-3') and 0.2 µM mcrA-R (5'-TTCATTGCGCTTGGTATAG-3') primers. Each mcrA PCR was as follows: an initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C denaturation for 35 s, 55 °C annealing for 45 s, 72 °C elongation for 2 min, followed by a final elongation at 72 °C for 2 min. After amplification, the PCR product was cleaned using a Purelink PCR clean up kit (Invitrogen) as per manufacturer’s instructions. After the clean-up, the PCR product was quantified using Invitrogen’s Qubit technology (Invitrogen).

Terminal restriction fragment length polymorphism (T-RFLP) PCR was run in an ep gradient pro realplex² master cycler (Eppendorf) and contained 1 µL of 1/100 diluted DNA sample from an incubated core, 1 × 1.5 mM MgCl₂ PCR buffer (Idaho Technology Inc.), 0.4 mM dNTPS, 1 unit of Taq, 0.2 µM mcrA-FAM-labelled (5'-GGTGGTGTGGAGATCACCACACGTTAG-3') and 0.2 µM mcrA-R (5'-TTCATTGCGCTTGGTATAG-3') primers. Each mcrA PCR was as follows: an initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C denaturation for 1 min, 53.5 °C annealing for 1.5 min, 72 °C elongation for 1.5 min, followed by a final elongation at 72 °C for 10 min. Each T-RFLP PCR was followed by a precipitation clean-up with ethanol. After a precipitation clean-up, 1× Buffer E (Promega, Madison, WI, U.S.A.) 5 units of TaqI restriction enzyme and 0.2 µL of 10 mg mL⁻¹ BSA were added to each purified FAM-labelled PCR product for a 25-µL total digestion volume. Enzyme digestion was performed at 65 °C for 2 h. Each digestion sample was then cleaned up with an ethanol precipitation, and the University of Notre Dame Genomics Core conducted fragment analysis with an ABI 3730xl DNA analyser (Applied Biosystems, Carlsbad, CA, U.S.A.).

Statistical analysis

One-way ANOVA was used to test the significance of our carbon addition treatments on CH₄ production rates.
post hoc test, Tukey’s multiple comparisons of means with a 95% family-wise confidence level, was performed to compare mean CH$_4$ production rates from carbon-amended cores. A two-way ANOVA was used to investigate the influence of our treatments and water versus sediment effects on mcrA log$_{10}$ gene abundance. Non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM) were used for visualisation and to test for treatment effects on methanogen community composition. Significance of the ANOSIM statistic was evaluated using a 1000 permutations of the pairwise Bray–Curtis dissimilarity matrix. All statistical analyses were conducted in the R statistical environment using the base and vegan packages (R Development Core Team, 2008).

**Results**

**Diamond Lake profile**

Diamond Lake is a seasonally stratified lake. However, during our sampling, the lake had experienced autumn turnover. Thus, we observed a relatively consistent dissolved oxygen and temperature profile (Fig. 1). Methane concentrations ranged from 5 to 7.6 µM from the surface to 11 m. A sharp increase in CH$_4$ concentrations was observed below 11 m (Fig. 1).

**CH$_4$ production rates**

Overall, we observed significant effects of carbon addition on CH$_4$ production rates (one-way ANOVA, $F = 32.5$, $P = 0.007$, d.f. = 2, 6). Algal cores had the highest CH$_4$ production rate during the incubation period and were significantly higher than control cores and those receiving terrestrial carbon (Fig. 2). The average CH$_4$ production rate of the terrestrial cores was higher than the control, but not significantly different (Fig. 2).

**Methanogen abundance and community composition**

All sediments and overlying water contained mcrA at the end of our incubations. Per-gram sediment concentrations of mcrA were significantly higher than overlying water mcrA concentration (per millilitre) by about 100-fold (two-way ANOVA, $F = 17.7$, $P = 0.001$, d.f. = 1, 12; Fig. 3). However, there was not a significant difference in mcrA gene copies between algal, terrestrial and control sediment cores in either water or sediment samples (two-way ANOVA, $F = 0.22$, $P = 0.80$, d.f. = 2, 12).

Terminal restriction fragment length polymorphism revealed nine different operational taxonomic units (OTU) in the incubated cores. Analysis of similarity revealed no effect of treatment on mcrA composition within sediment samples (ANOSIM, $R = 0.05$; $P = 0.35$). A single mcrA OTU was observed in all overlying water samples.
amendments. The lack of change in methanogen community composition amongst algal and terrestrial cores may result from the limited diversity of metabolic substrates used by methanogens (primarily acetate and H₂/CO₂) (Deppenmeier, Müller & Gottschalk, 1996; Roy & Conrad, 1999). The fermentative processes that produce these precursors may negate the differences between algal and terrestrial carbon. Thus, it may be that changes in community composition occur at the level of the fermentative bacterial community and not within methanogen communities. Further experimentation is needed to investigate the role of fermenter community composition in controlling substrate availability for methanogens.

A similar increase in CH₄ production was observed in cores and slurries amended with algal biomass of Peridinium gatunense Nygaard 1925 from Lake Kinneret (Schwarz, Eckert & Conrad, 2008). However, their data suggest that 16s rRNA-based community composition changed significantly after 6 days of incubation with additional algal carbon. It is possible that differences in methodology can account for the apparent disagreement between our results. For example, we carried out qPCR and T-RFLP based on the mcrA gene, whereas their T-RFLP was carried out using 16s rRNA. These two loci may provide different taxonomic resolutions. However, previous studies have demonstrated that mcrA is a sensitive genetic marker for methanogen community composition and phylogenetic studies (Luton et al., 2002; Earl et al., 2003; Milferstadt et al., 2010). In addition, the use of general bacterial and archaeal primers by Schwarz et al. (2008) seems to have detected changes in non-methanogenic components of the community, perhaps including fermenters. Nonetheless, both the Schwarz study (2008) and our findings confirm that CH₄ production increases with algal amendments, and both studies suggest that eutrophication of lakes on regional and global scales may contribute to the global CH₄ budget. However, we suggest that shifts in methanogen community composition and abundance (Fig. 2) do not account for increased methanogenesis rates.

Many bacterial communities preferentially utilise more labile algal carbon than recalcitrant terrestrial carbon because it promotes higher growth efficiencies (Kritzberg et al., 2004, 2005). Additionally, other work has demonstrated the influence of resource quality on CH₄ production in sediments (Kankaala, Kaki & Ojala, 2003). A rapid response of CH₄ production to algal carbon addition probably reflects the high lability of algal biomass (Updegraff et al., 1995). The relatively recalcitrant nature of terrestrial carbon may lead to slower mineralisation by fermenters and a reduced, but perhaps extended, metha-
nogenesis response. Our data are in line with the possibility of this hypothetical dichotomy in lability and timing of enhanced methanogenesis whilst using algal and terrestrial carbon substrate. Labile algal carbon was readily fermented, resulting in significant increases in methanogenesis over the 2.5-week incubation. In comparison, terrestrial carbon sustained a lower increase in methanogenesis that may have persisted for a longer period of time. Alternatively, fermenters may utilise terrestrial carbon at a lower efficiency, and subsequently, a lower supply of substrate may be available for methanogens to use. In future, extended incubation periods would enable a test of this interesting hypothesis.

Overall, our study suggests that algal carbon sources are readily fermented into substrates that are reduced by methanogens, resulting in an increase in CH4 production. Consequently, eutrophication may lead to higher CH4 production rates on a whole-lake scale. Globally, more lakes are becoming vulnerable to the effects of anthropogenic nutrient loading (Smith, 2003), and eutrophication could play a major role in global CH4 budgets. However, further investigation, at appropriate scales of inference, is needed to account for complex feedback that can be limited in microcosm studies. Future studies should focus on eutrophication and humic state dynamics within lakes on larger, and potentially longer, scales to examine the effects of nutrients and terrestrial DOC directly as well as indirectly on microbial biogeochemical interactions, CH4 production and net CH4 flux.

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