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Author(s): Hiltunen, Minna; Nykänen, Hannu; Syväranta, Jari

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understanding the processes involved in sediment CH4

The main production pathways for CH₄ during anoxic degradation of OM in freshwater sediments are acetoclastic methanogenesis (acetate as precursor) and hydrogenotrophic methanogenesis (H₂ and CO₂ as precursors) (Whiticar et al. 1986; Conrad 2020). The relative importance of these pathways is determined by temperature, microbial community composition, and the characteristics of the substrate, which influences the production rates of acetate, H₂, and CO₂ during the initial hydrolysis and fermentation step of complex organic compounds (Conrad 2020). Analysis of stable isotope

The influence of lipid content and taxonomic affiliation on methane and carbon dioxide production from phytoplankton biomass in lake sediment

Minna Hiltunen ^(D),^{1,2*} Hannu Nykänen,³ Jari Syväranta^{1,3}

¹Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland ²Biological and Environmental Science. University of Ivväskylä. Ivväskylä. Finland ³Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland

Abstract

The greenhouse gases methane (CH_4) and carbon dioxide (CO_2) are end products of microbial anaerobic degradation of organic matter (OM) in lake sediments. Although previous research has shown that phytoplankton lipid content influences sediment methanogenesis, current understanding on how OM quality affects methanogenesis is still limited. Such information is needed to more accurately assess how lake greenhouse gas emissions may change in response to anthropogenic activities. We cultured 11 phytoplankton species from five classes and studied how taxonomic identity, C : N ratio, lipid content, and fatty acid composition of phytoplankton biomass affects the CH₄ and net CO₂ production in anaerobic lake sediments with an incubation experiment that lasted > 100 d. The carbon-normalized potential CH₄ (0.09–0.23 μ mol mg C⁻¹ d⁻¹) and net CO_2 (0.09–0.28 µmol mg C⁻¹ d⁻¹) production rates were not related to phytoplankton taxonomic affiliation (e.g., class, species), C : N ratio, or fatty acid composition of algal biomass. Methane or net CO_2 production potentials did not increase with higher lipid content (10–30%); however, total fatty acid content had a weak correlation with CH₄ production potential. In contrast to previous research, our results suggest that lipid content is of minor importance in determining methanogenesis rates from the biomass of multispecies phytoplankton communities settling on sediments. The decrease in CO_2 concentration and the correlation between stable carbon isotope signatures of CH₄ and molar ratio of CH₄ and CO₂ at the end of the experiment may indicate that importance of hydrogenotrophic methanogenesis, which uses CO_2 when other substrates become limiting, increased during the long incubation.

Methane (CH₄) and carbon dioxide (CO₂) production in lake sediments and subsequent emission into the atmosphere are important processes in global carbon cycle (Cole et al. 2007; Bastviken et al. 2011). On a timescale of 100 yr, the Global Warming Potential of CH₄ is 28 times that of CO₂ (IPCC 2014). Furthermore, CH₄ emissions from boreal lakes are predicted to increase considerably due to warming climate and longer ice-free seasons (Wik et al. 2016), and thus

*Correspondence: minna.m.hiltunen@jyu.fi

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production is crucial. Methane is the final product of anaerobic microbial decomposition of organic matter (OM) when oxygen and alternative electron acceptors, for example, nitrate (NO_3^-) , sulfate (SO_4^{2-}) , and iron (Fe^{3+}) , are used (Capone and Kiene 1988; Conrad 2020). Sediment temperature, extent of anoxia, pH, and substrate quantity and quality are all thought to regulate the rates of methanogenesis in lake sediments (Duc et al. 2010; West et al. 2015).





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ratios of carbon (δ^{13} C) in produced CH₄, CO₂, and precursors of methanogenesis is used for resolving which pathway dominates the CH₄ production (Conrad 2005). However, the estimation of pathways is rather difficult due to variability between microbial taxa and environmental conditions in isotopic fractionation of carbon during the OM degradation processes (Conrad 2005; Goevert and Conrad 2009; Heuer et al. 2010).

There is a link between epilimnetic primary production and sediment processes as 10–50% of algae in the mixed layer settles to sediment surface (Baines and Pace 1994). Furthermore, laboratory experiments have demonstrated that algae are quickly converted into suitable substrates for methanogenesis (Schulz and Conrad 1995; Schwarz et al. 2008). Methanogenesis in freshwater sediments increases with inputs of phytoplankton biomass (West et al. 2012, 2015; Grasset et al. 2018) and algae are considered a better substrate for methanogenesis compared to more recalcitrant allochthonous OM (Schwarz et al. 2008; West et al. 2012; Davidson et al. 2015).

Phytoplankton taxa vary in their elemental and biochemical composition (Brown et al. 1997; Peltomaa et al. 2017), which may affect degradation rates in lake sediments. Low carbon to nitrogen (C : N) ratios in sediment (< 10), indicating input from primary production, are associated with high potential CH₄ production rates (Duc et al. 2010), where high C : N ratios indicate that OM is rich in complex compounds such as polysaccharides or lignin and that N might be limiting for microbial degradation (Enríquez et al. 1993). Total phosphorus enhances the aerobic degradation of recalcitrant allochthonous OM, indicating that degradation rates are determined by the interactions of biochemical composition of the substrate and nutrients (Guillemette et al. 2013). Furthermore, the nutrient stoichiometry and the biochemical composition, especially the lipid and protein content, of phytoplankton will change in response to nutrient limitation (Shifrin and Chisholm 1981; Sterner and Hessen 1994; Rodolfi et al. 2008).

Phytoplankton lipid content (per dry weight) varies from 5% to 70% of biomass depending on the species, strain, and growth conditions (Shifrin and Chisholm 1981; Brown et al. 1997; Rodolfi et al. 2008). In general, nutrient stress increases the lipid content of phytoplankton (Shifrin and Chisholm 1981; Rodolfi et al. 2008). Theoretical methane yield in anaerobic digestion is higher from lipids than from proteins or carbohydrates, and thus biomass grown with nutrient limitation has a higher theoretical CH₄ yield potential (Symons and Buswell 1933; Angelidaki and Sanders 2004; Labatut et al. 2011). The importance of substrate quality on the rates of methanogenesis in lake sediments is not yet well understood; however, West et al. (2015) found that phytoplankton lipid content has a positive effect on methanogenesis, in line with the estimates from theoretical methane yield potentials. Furthermore, methanogenesis was similar with *Scenedesmus obliquus* and *Microcystis aeruginosa*, a green algae and cyanobacteria, when lipid content was held constant, suggesting that lipid content has a greater effect on methanogenesis than other biochemical or structural differences among phytoplankton taxa (West et al. 2015). Polyun-saturated fatty acids in phytoplankton biomass were degraded faster in anoxic conditions than saturated fatty acids (Harvey and Macko 1997; Grossi et al. 2001), suggesting that in addition to lipid content, lipid composition might have an effect on the rate of methanogenesis. Very high concentrations of long-chain fatty acids may inhibit methanogenesis in engineered systems (Lalman and Bagley 2000; Cirne et al. 2007), but it is unlikely that this would happen in sediments where OM loading is much lower.

While nutrient stress may greatly modify the lipid content of algae, the composition of fatty acids is rather stable, and determined by phylogeny (Galloway and Winder 2015). The chain-length and degree of saturation of fatty acids varies significantly among phytoplankton classes and is an important factor determining the nutritional quality of phytoplankton to consumers (Galloway and Winder 2015; Peltomaa et al. 2017). However, environmental factors, such as nutrient concentrations, may greatly modify both the biomass and community composition of phytoplankton in lakes (Tilman et al. 1982). Therefore, phytoplankton community composition will change in response to increases in nutrient input (eutrophication) but also water color (browning), which both can be driven by anthropogenic actions and climate change (e.g., Moss et al. 2011; Kritzberg et al. 2020).

The view that lakes are significant global sources of greenhouse gases has been strengthened by recent research (Bastviken et al. 2011; Davidson et al. 2018; Beaulieu et al. 2019) making the mechanisms and controls of methane production in lakes an important and timely topic. A comprehensive test of how taxonomic identity, and lipid content composition of phytoplankton biomass affects and methanogenesis rates in lake sediments is still lacking. To address this question, we conducted a laboratory incubation experiment with 11 phytoplankton species with varying lipid content and composition, and followed the production of CH₄ and CO₂ in the anaerobic lake sediment with the addition of algal biomass mixtures for over 100 d. The length of the experiment represents typical anoxic periods that may take place in sediment surface or hypolimnetic water layers of dimictic boreal lakes in this region during winter ice-cover or summer stratification. Also, the duration of the experiment allows to fully examine the gas production fueled by the decomposition of labile compounds in phytoplankton. Our hypotheses were that phytoplankton (1) lipid content is positively correlated with CH₄ and net CO₂ production potentials, (2) fatty acid composition (dependent on phylogeny) does not affect CH₄ and net CO₂ production potentials. Furthermore, we analyzed stable isotopes of carbon in the substrate and the produced CH₄ and CO₂ in order to study

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the potential production pathways for CH₄ in late phase of the experiment.

Materials

Phytoplankton cultures

To study the effect of taxonomic identity, lipid content, and composition of phytoplankton biomass on CH₄ and CO₂ released during degradation in lake sediment, we cultured 11 phytoplankton species from five different classes in the laboratory. We grew three diatom species (Nitzschia sp., Navicula sp., Fragilaria sp.), four green algae (Acutodesmus sp., Monoraphidium griffithii, Chlamydomonas sp., Selenastrum sp.), two cyanobacteria (Microcystis aeruginosa, Pseudanabaena tremula), one cryptophyte (Cryptomonas sp.) and one chrysophyte (Mallomonas kalinae). The phytoplankton were grown at 20°C in 2-3 L Erlenmeyer flasks with 16 : 8 h light : dark cycle. The growth medium was Z8 for cyanobacteria, green algae, and Cryptomonas (Staub 1961) and WC for diatoms, and Mallomonas (Guillard and Lorenzen 1972). Algae were grown for 2-4 weeks to produce a dense culture and then half of the volume was harvested by centrifugation (high N treatment). Then, the harvested volume was replaced with fresh media with no N (e.g., producing ca. half the N concentration of the high N treatment) and the algae were grown further 2-4 weeks with low N before harvesting. Phosphorus concentration in the cultures was not changed. The nutrient treatments were aimed to modify the lipid and fatty acid content of the algal biomass without affecting the fatty acid composition. The phytoplankton biomass was stored in a freezer and freeze-dried prior the experiment.

Incubation experiment to estimate potential production rates of CH₄ and CO₂

Surface sediment used in the incubations was collected with an Ekman Dredge from the ice-covered Sompalampi pond ($62.62^{\circ}N 29.52^{\circ}E$) in spring 2019. Water depth at sediment collection point was 5 m. In addition to sediment, we also sampled water from the anoxic hypolimnion. Water and sediment were stored at +4°C before the experiment. In laboratory, we mixed 1439 g of sediment and 975 g of hypolimnetic water, and added 50 mL of this slurry to 47250 mL laboratory flasks (Schott Duran, Germany). Freezedried aliquots (~ 10 mg dry weight) of 11 different algal species grown in low and high N treatments (n = 1-3) were added to the bottles except to three used as controls.

Bottles were subsequently capped with butyl rubber septum (Massive black butyl stopper for GL 45 flask) secured by open top screw caps (Schott). Algae and sediment slurries were mixed with Vortex for 1 min. The remaining 200 mL head-space was then vacuumed and filled three times with 99.999% N_2 gas to ensure anoxic conditions. We left 1 atm overpressure in the bottles in order to ensure overpressure for gas analyses. The slurries were incubated at 10°C in the dark for 136 d (LMS

cooled incubator, GB). On days 16, 30, 45, 60, 73, 86, 116, and 136, a 25 mL gas sample was extracted from each slurry headspace. Slurries were only briefly shaken before gas measurements to remove bubbles, as mixing can affect methanogenesis (Dannenberg et al. 1997). Gas samples (25 mL) were injected into a 12 mL prevacuumed exetainer vials for CH_4 and CO_2 quantification with gas chromatography.

Gas samples for days 16-116 were analyzed with an Agilent 6890 Gas Chromatograph equipped with Flame ionization detector (FID) for CH₄ and Thermal conductivity detector (TCD) for CO₂. Gas concentrations were quantified with laboratory standards of CH₄ and CO₂. In day 136 samples, the concentration of CH₄ and CO₂ was measured with Picarro 2201-I (Picarro, Sunnyvale, California, U.S.A.) analyzer. However, these incubation flasks were accidentally frozen (and then thawed) prior to analysis, and the concentration data of these measurements were not used in CH₄ and net CO₂ production calculations. We chose to report CH₄ and CO₂ production as the sum of headspace and water-phase CO₂ taking into account Henry's law. In CO2 calculation, we excluded dissolved carbonates as all treatments were incubated in the same slightly acidic sediment slurry. Similar to Grasset et al. (2019), this is a conservative measure of CO₂ production during degradation. The amount of CH₄ and CO₂ produced in bottles without algae was subtracted from the values in bottles with algae to account for gas production from the degrading sediment alone. The progressive decrease in gas volume due to sampling was taken into account in the calculations. The amount of CH₄ and CO₂ gas moles removed in each sampling point was calculated from measured concentrations, and the corresponding amount of CH₄ or CO₂ was added to produced gas amounts in the next sampling point. Linear regression model between days 30 and 86 was used for potential CH₄ production calculations, except for four bottles, in which the linear phase was somewhat shorter and data for days 30-75 or 45-86 were used for the calculations. Net CO₂ production was estimated between days 16 and 45, since before day 16 the production was most likely due to other processes than methanogenesis and the CO₂ concentration increase leveled off after day 45 (Fig. 1). In one bottle with Fragilaria, the CH₄ production started only after day 75, potentially due to oxygen contamination in the beginning, and it was omitted from figures and any further analysis.

We measured pH and electrical conductivity in the slurries at the end of the experiment (day 136) with WTW pH 340 with WRW SenTix 81 pH electrode and WTW pH/cond 341 with aCon 325 electrode.

Lipid and fatty acid analyses of phytoplankton biomass

Lipid and fatty acid content and composition was analyzed from freeze-dried algal biomass. The lipids were extracted twice with 2 : 1 chloroform-methanol (by volume) aided with ultrasonication. The extract was concentrated and split between analysis of lipids and fatty acids. The amount Hiltunen et al.



Fig. 1. CH_4 and CO_2 accumulation in bottles where algae was added (mean \pm SD, n = 43) and in control bottles without algal addition (n = 3). CH_4 production potentials (μ mol CH_4 d⁻¹ mg C⁻¹) were calculated for period of day 30–86 (*see* "Materials" section for exceptions) when the increase was linear. Correspondingly, net CO_2 production potentials (μ mol CO_2 d⁻¹ mg C⁻¹) were calculated for days 16–45.

of lipids was measured gravimetrically by evaporating off the solvent in preweight tin cups and weighing the remaining lipids with a microbalance. For the analysis of fatty acids, we produced fatty acid methyl esters with acid-catalyzed transesterification (H₂SO₄ in methanol) while keeping the samples in heat block (at 90°C) for 90 min. The samples were dissolved in *n*-hexane and run with a gas chromatograph-mass spectrometer (GC-MS, Agilent 6890 and 5973N, Santa Clara, California, U.S.A.). Samples were injected splitless at 250°C. The column was DB-23 (Agilent, 015 μ m × 0.25 mm × 60 m) and the average velocity of the carrier gas (helium) was 23 cm s⁻¹. The initial oven temperature was 50°C, and after 1 min the temperature was raised 15° C min⁻¹ to 150° C, then 1.5°C min⁻¹ to 160°C, 1.0°C min⁻¹ to 170°C, and finally 1.5°C min⁻¹ to 230°C. Peaks were identified using mass spectra and retention times of a standard fatty acid mix (GLC-538, Nu chek prep., Elysian, Minnesota, U.S.A.), which was also used for correcting the MS response. The saturated fatty acid 21:0 was used as the internal standard. The extraction of lipids mobilizes also other compounds from the biomass, for example, pigments, which was clearly seen in our phytoplankton samples. The sum of fatty acids per dry weight (μ g FA mg DW⁻¹) excludes solvent-extracted compounds that do not contain fatty acids, and may better represent the "true" lipid compounds. Thus, we chose to report both the proportion of lipids per DW and the sum of fatty acids to get a better picture on how lipids influence methanogenesis.

Stable isotope analysis of biomass, sediment, and CH₄

Stable isotope composition and C% and N% of freeze-dried algae was analyzed with a Thermo Finnigan Advantage IRMS (San Jose, California, U.S.A.) coupled with the elemental analyzer FlashEA 1112 (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.). Sompalampi sediment δ^{13} C was analyzed from oven dried (65°C, 48 h) samples. Acid fumigation before analyses did not affect sediment δ^{13} C values. Stable isotope composition was expressed in the delta notation as a ‰ deviation of the heavy-to-light isotope abundance ratio in the sample from that of a standard, Vienna PD belemnite:

$$\delta^{13}C = \left(\frac{\left(\frac{13}{12}\right)sample}{\left(\frac{13}{12}\right)standard} - 1\right) * 1000$$
(1)

For C% and N%, certified birch leaf standard (Elementar Microanalysis, UK) was used as a reference, which was also used as in-house standard for δ^{13} C and δ^{15} N analyses.

Stable isotopes of CH₄ (δ^{13} CH₄) and CO₂ (δ^{13} CO₂) and the concentration of CH₄ and CO₂ were measured from day 136 samples with Picarro 2201-I analyzer. A standard with known stable isotopic composition of carbon in CH₄ and CO₂ (Air Liquide, Alphagaz) was used for calibration of sample δ^{13} CH₄ and δ^{13} CO₂ values. In order to control linearity of δ^{13} CH₄ and δ^{13} CO₂ values in each run, the injection volumes were adjusted to correspond to concentration of the used standard.

Statistics

t-Test was used for testing intraspecific differences in lipids (%) and total fatty acids between the two N treatments. Variation in fatty acid percent composition of phytoplankton species was illustrated with using the unconstrained ordination method nonmetric multidimensional scaling. Permutational multivariate analysis of variance (PERMANOVA) with type III sums of squares and permutations of residuals under a reduced model was used for detecting differences in fatty acid percent composition among phytoplankton classes, species, and N treatments (high/low). Class and N treatment was handled as fixed factors and species as a random factor nested under the factor class. Multivariate analyses were conducted on untransformed data using Euclidean distance as the dissimilarity measure. Differences in CO2 and CH4 production potentials among phytoplankton classes and species was tested with nonparametric Kruskal-Wallis test, because the assumptions for ANOVA were not met. The connection between gas production and phytoplankton biomass quality (C : N ratio, lipid content) was examined with Pearson correlation coefficients (r), while due to outliers, Spearman correlation coefficients were used for total fatty acid content and gas production. We used linear regression to investigate the relationship between the molar ratio of CH₄ and CO₂ and the δ^{13} CH₄ and δ^{13} CO₂. We used IBM SPSS 24 and PRIMER 7 with PERMANOVA+ addon for statistical testing.

Results

Phytoplankton biomass

The molar C : N ratios of algal biomass were between ca. 2 and 11, and in general, the C : N ratio increased when N was limiting (Table 1). The sediment used in experiments contained 32.7% of carbon and 2.2% of nitrogen; hence the molar C : N ratio was 17.3.

The lipid content (%DW) and the fatty acid content of phytoplankton biomass were strongly correlated (r = 0.887, p < 0.001, n = 36). The N treatments were not drastic enough to produce large difference in C : N, or algal lipid and fatty acid content (Table 1). Only in Chlamydomonas, Fragilaria, Pseudanabaena, and Microcvstis, the low N treatment had significantly higher lipid content than the high N treatment (t-test, t = 3.834-14.343, p < 0.02), and even in those the difference was quite small (Table 1). However, among the algal species the lipid content varied from 10% to 30% of DW, and total fatty acid content from ca. 25 to 180 μ g mg DW⁻¹. The factor class explained the most variation in phytoplankton fatty acid percent composition (PERMANOVA, $F_{4.40} = 12.05$, p < 0.001, $R^2 = 0.82$, Supporting Information Fig. S1, Table S1), and all classes differed from each other in terms of fatty acid composition (pairwise comparisons, t = 4.239-19.773, p < 0.005). Minor part of the variation was related to species (nested under class, $F_{6.45} = 178.35$, p < 0.001, $R^2 = 0.15$) and the interaction of the factors species and N treatment ($F_{6,45} = 12.66$, p < 0.001, $R^2 = 0.02$), but as expected, class identity was the major determinant of phytoplankton fatty acid composition.

Methane and carbon dioxide production potentials

Carbon dioxide production started rapidly after the start of the experiment and was higher in the bottles where algae were added than in control bottles (Fig. 1). However, the CO₂ production rate started to level off after day 45. Methane concentrations started to increase after 16 d of incubation, and CH₄ production without phytoplankton additions was only a small fraction of that when algae was added (Fig. 1). Twenty-one to forty-seven percentage of the added algal carbon was degraded during 116 d of incubation as calculated from the produced CH₄ and CO₂ (Table 2). The CH₄ or net CO₂ production potentials (as μ mol d⁻¹ mg C⁻¹) did not differ among algal classes (Fig. 2, Kruskal–Wallis, $\chi^2 = 6.185$, p = 0.186, n = 43, and $\chi^2 = 5.382$, p = 0.250, n = 43, for CH₄ and CO₂, respectively). However, there were some differences among the phytoplankton species in CH₄ ($\chi^2 = 19.049$, p = 0.040, n = 43, Fig. 2) and net CO₂ production potentials ($\chi^2 = 20.285$, p = 0.027, n = 43), but the Bonferroni-corrected pairwise tests did not detect significant differences, indicating that the differences were small and/or the number of replicates too low. Thus, differences in CH₄ or net CO₂ production potentials were not explained by phylogenetic relationships of the phytoplankton (e.g., class/species), which suggests that fatty acid composition (e.g., the chain-lengths and degree of saturation), which was highly dependent on phytoplankton class (see above), did not affect gas production from degrading phytoplankton biomass.

In the algal species where N treatments produced differences in lipid or total fatty acid content, the potential CH_4

Table 1	Lipid,	and total	fatty aci	id content (FA,	mean \pm SD,	n = 1–3),	and mola	ar C : N	ratio of	phytoplankton	biomass	used i	n the
incubatio	n exper	iment.											

	C :	N	Lipio	d (%)	Tot. FA (μ g mg DW ⁻¹)		
Таха	High N	Low N	High N	Low N	High N	Low N	
Green algae							
Chlamydomonas sp.	6.7	6.8	13.3 ± 1.5	$\textbf{20.6} \pm \textbf{0.6}^{*}$	40.3 ± 1.8	$60.7\pm8.7^{*}$	
Monoraphidium griffithii	5.0	6.7	$\textbf{23.8} \pm \textbf{1.2}$	$\textbf{24.4} \pm \textbf{1.9}$	$\textbf{82.8} \pm \textbf{12.4}$	$\textbf{99.2} \pm \textbf{10.9}$	
Scenedesmus sp.	5.9	7.8	14.9 ± 1.2	14.6 ± 1.6	$\textbf{35.3} \pm \textbf{0.7}$	$\textbf{32.4} \pm \textbf{1.9}$	
Selenastrum sp.	5.4	7.5	17.5 ± 1.9	19.3 ± 0.7	$\textbf{36.4} \pm \textbf{1.2}$	$\textbf{57.0} \pm \textbf{2.6}^{*}$	
Diatoms							
Fragilaria sp.	5.8	8.3	10.9 ± 0.0	$15.9\pm0.9^{*}$	61.3 ± 2.7	$88.5 \pm 5.5^{*}$	
Navicula sp.	7.3	7.8	$\textbf{24.3} \pm \textbf{1.9}$	$\textbf{24.2} \pm \textbf{1.0}$	145.6 ± 10.9	137.7 ± 10.6	
Nitzschia sp.	10.6	10.1	30.0	26.7 ± 0.4	178.8 ± 7.5	$148.1\pm8.4^{*}$	
Cryptophytes							
Cryptomonas sp.	4.5	4.7	15.5 ± 0.5	17.1 ± 1.7	$\textbf{50.9} \pm \textbf{3.4}$	$\textbf{57.3} \pm \textbf{7.7}$	
Chrysophytes							
Mallomonas kalinae	4.9	4.8	11.7 ± 0.9	11.5 ± 0.5	25.3 ± 0.5	25.7 ± 0.2	
Cyanobacteria							
Microcystis aeruginosa	5.2	5.7	10.1 ± 0.4	$11.3\pm0.4^{*}$	$\textbf{25.3} \pm \textbf{3.2}$	$\textbf{33.8} \pm \textbf{3.0}^{*}$	
Pseudanabaena tremula	2.4	4.7	10.2 ± 0.2	$13.0\pm0.3^{\star}$	$\textbf{35.5} \pm \textbf{1.6}$	$\textbf{48.0} \pm \textbf{8.0}$	

*Significant differences in lipid (%) or total fatty acid content between high and low N treatments (*t*-test, p < 0.01).

Table 2. Proportion of added algal C degraded in 116 d, and potential CH₄ and net CO₂ production (μ mol d⁻¹ mg C⁻¹) during linear phase of increase (days 30–86 for CH₄ and days 16–45 for CO₂) of high and low N treatments of different algal species (mean \pm SD, n = 1-4) and in the control bottles (n = 3).

	CH₄ (µmol d	$^{-1}$ mg C $^{-1}$)	CO ₂ (µmol d	$^{-1}$ mg C $^{-1}$)	Prop. of added C degraded (%)		
Таха	High N	Low N	High N	Low N	High N	Low N	
Green algae							
Chlamydomonas sp.	$\textbf{0.14} \pm \textbf{0.03}$	$\textbf{0.13} \pm \textbf{0.03}$	$\textbf{0.14} \pm \textbf{0.01}$	$\textbf{0.14} \pm \textbf{0.01}$	25.0	25.9	
Monoraphidium griffithii	$\textbf{0.18} \pm \textbf{0.01}$	$\textbf{0.14} \pm \textbf{0.00}$	$\textbf{0.12} \pm \textbf{0.04}$	$\textbf{0.15} \pm \textbf{0.03}$	37.2	29.6	
Scenedesmus sp.	$\textbf{0.14} \pm \textbf{0.03}$	$\textbf{0.13} \pm \textbf{0.03}$	$\textbf{0.15} \pm \textbf{0.01}$	$\textbf{0.13} \pm \textbf{0.00}$	28.8	24.2	
Selenastrum sp.	$\textbf{0.18} \pm \textbf{0.00}$	$\textbf{0.09} \pm \textbf{0.00}$	$\textbf{0.19} \pm \textbf{0.02}$	$\textbf{0.17} \pm \textbf{0.00}$	32.3	22.9	
Diatoms							
Fragilaria sp.	$\textbf{0.12} \pm \textbf{0.03}$	0.14	$\textbf{0.22} \pm \textbf{0.00}$	0.28	28.9	35.0	
Navicula sp.	$\textbf{0.22}\pm\textbf{0.01}$	0.22	$\textbf{0.17} \pm \textbf{0.00}$	0.13	39.3	43.6	
Nitzschia sp.	$\textbf{0.18} \pm \textbf{0.01}$	$\textbf{0.18} \pm \textbf{0.01}$	$\textbf{0.14} \pm \textbf{0.01}$	$\textbf{0.15} \pm \textbf{0.01}$	34.6	32.5	
Cryptophytes							
Cryptomonas sp.	0.15	$\textbf{0.17} \pm \textbf{0.01}$	0.14	$\textbf{0.17} \pm \textbf{0.01}$	28.1	31.9	
Chrysophytes							
Mallomonas kalinae	0.12	0.16	0.19	0.22	28.0	33.8	
Cyanobacteria							
Microcystis aeruginosa	$\textbf{0.13} \pm \textbf{0.04}$	$\textbf{0.15} \pm \textbf{0.01}$	$\textbf{0.15} \pm \textbf{0.04}$	$\textbf{0.16} \pm \textbf{0.02}$	29.4	30.4	
Pseudanabaena tremula	$\textbf{0.23} \pm \textbf{0.07}$	0.11	$\textbf{0.23} \pm \textbf{0.05}$	0.09	46.8	20.6	
Control	$0.003\pm0.002^{\star}$		$\textbf{0.167} \pm \textbf{0.027}^{*}$		n.a.		

n.a., not applicable.

 $^{*}\mu$ mol d⁻¹.

(or CO_2) production did not increase with higher lipid content of the biomass (Table 2, Supporting Information Fig. S2). When all the data was pooled, lipid content of algal biomass did not correlate with CH₄ production potentials (Fig. 3; r = 0.188, p = 0.228, n = 43), but net CO₂ production potentials had a weak, negative correlation with lipid content of algae (r = -0.353, p = 0.020). The total fatty acid content correlated weakly with CH₄ production potentials (Supporting Information Fig. S3, Spearman correlation, r = 0.319, p = 0.037, n = 43) but not with net CO₂ production potentials (p = 0.177). Furthermore, the CH₄ and net CO₂ production potentials (as μ mol d⁻¹) correlated with the amount of C added to bottles (r = 0.447, p = 0.003, n = 43, and r = 0.337, p = 0.027, respectively). CH₄ production potentials did not correlate with molar C : N ratio or the amount of added N in phytoplankton biomass (p > 0.05). Net CO₂ production potentials had a weak, negative correlation with molar C : N ratio (r = -0.324, p = 0.034, n = 43), but not with the amount of added N in phytoplankton biomass (p = 1.000).

Stable isotopes of algal biomass and produced methane

The δ^{13} C values of phytoplankton biomass varied from ca. –16 to –33‰ and δ^{15} N values from –11 to 7‰ (Supporting Information Table S2). The δ^{13} C of sediment was –28‰. The δ^{13} C of produced CH₄ and CO₂ in the end of the experiment

correlated with the δ^{13} C of phytoplankton biomass, but this relationship was largely driven by low values in the chrysophyte *Mallomonas* (Supporting Information Fig. S4, r = 0.565, p < 0.001, n = 43, and r = 0.752, p < 0.001, for CH₄ and CO₂, respectively). Variation in δ^{13} C of produced CH₄ (linear regression: y = 26.93x - 78.846, $R^2 = 0.787$, $F_{1,41} = 151.494$, p < 0.001, Supporting Information Fig. S5) and CO₂ (y = 14.01x - 16.524, $R^2 = 0.426$, $F_{1,41} = 30.407$, p < 0.001) was explained by the molar ratio of CH₄ and CO₂ at the end of the experiment.

pH and conductivity at the end of the experiment

At the end of the experiment, pH was 6.24 ± 0.04 (6.17–6.30) (mean \pm SD [range]) in control flasks and statistically significantly higher (t = 6.793, p < 0.001, df = 44) in the bottles where phytoplankton was added (6.64 ± 0.10 [6.45–7.04]). Conductivity was 66 ± 10 mS cm⁻¹ (59–78) in control bottles and 69 ± 15 mS cm⁻¹ (47–111) in bottles where algae was added.

Discussion

The results from our incubation experiment are in line with earlier studies showing that OM additions to lake sediments increase CH_4 production rates (Schwarz et al. 2008; West et al. 2012, 2015; Grasset et al. 2018, 2019). Furthermore, earlier studies show that CH_4 production from lake sediments



Fig. 2. CH_4 and net CO_2 production potentials of phytoplankton biomass incubated in lake sediment (n = 2-6).

increases with algal biomass input (West et al. 2012; Grasset et al. 2018), and field surveys have reported positive relationships between lake primary production and CH₄ production (West et al. 2016) and emissions of CH₄ (Deemer et al. 2016). Very little CH₄ was produced in control bottles without added phytoplankton biomass in our experiment, which demonstrates that sediment used in the incubations was very recalcitrant, and hence methanogenesis was substrate limited, likely due to exhausted substrates and negligible input of fresh OM during winter. CO₂ production was prominent in control bottles, indicating that part of the net CO₂ production also in bottles with algae likely was from the sediment, potentially confounding the effect of differences in quality of the algal substrate. Roughly a third of the added algal carbon was transformed to CO₂ and CH₄ in 116 d.

Carbon-normalized potential CH_4 or net CO_2 production rates were not positively connected to lipid content or composition of phytoplankton substrate in our sediment incubation experiment, in contrast to our first hypothesis. However, we did find a weak correlation with total fatty acid content and CH_4 production potential (but not with CO_2). Thus, it seems that lipids (or fatty acids) may have some influence on methanogenesis, but the effect was significantly weaker than in a previous study, which found higher lipid content of phytoplankton to enhance CH_4 production rates of sediment (West et al. 2015). Furthermore, CH_4 production in an



Fig. 3. The relationship between CH_4 or net CO_2 production potential and lipids (%) of phytoplankton biomass incubated in lake sediment. r = Pearson correlation coefficient. *p < 0.05. n.s., not significant.

engineered system (biogas reactor) increased with higher lipid content of the substrate (Zhao et al. 2014). However, both of these studies were much shorter (ca. 30 d) than our experiment (> 100 d).

Lipid content of phytoplankton varies greatly when cultured as monocultures in the laboratory (Shifrin and Chisholm 1981, Rodolfi et al. 2008), but the extent of variability in lakes with mixed communities is unclear. The range in biomass lipid content in the study of West et al. (2015) was somewhat narrower (14-28%) than in the present study (10-30%), but they found that doubling of lipid content yielded almost double the amount of methane from the green algae Scenedesmus (West et al. 2015). In our study, there was no positive correlation between production rates and biomass lipid content and only a weak correlation with total fatty acid content. Furthermore, there seemed to be no differences in CH₄ or net CO₂ production potentials in algae with intraspecific differences in lipid or total fatty acid content, in contrast to our first hypothesis. However, the differences in lipid content within algal species were smaller in our study than in the green algae studied by West et al. (2015). The range in lipid content of five phytoplankton species (from three classes) in the study of Zhao et al. (2014) in an engineered system was 2–37% (fatty acid methyl esters per ash free DW), with the lower end values produced by solvent extraction of phytoplankton biomass, and they found lipid content to explain large part of the variation in CH₄ production rates. Our gradient in lipid content included 11 taxa (from five classes) cultured in different nutrient regimes, and it is possible that some other parameter (e.g., protein content, elemental composition, etc.) that we did not measure confounded the potential effect of lipid content on CH₄ production rates observed in other studies. However, the phytoplankton communities in lakes contain dozens of species with varying nutrient and biochemical content, and our study suggests that lipid content does not determine CH₄ or CO₂ production rates in natural lake sediments.

Phylogenetic affiliation was not related to CH₄ or net CO₂ production potentials in our study with 11 species of phytoplankton, which corroborates the results from previous studies using lower number of taxa (Zhao et al. 2014; West et al. 2015). This indicates that fatty acid composition of lipids, which is class dependent and varied greatly among the phytoplankton taxa in our study, does not influence CH₄ or CO₂ production rates in sediment, consistent with our second hypothesis. Previous studies have reported contrasting results on anoxic degradation of phytoplankton lipids. Degradation rates of lipids from two species of marine phytoplankton were similar (Harvey et al. 1995), while another study found that monounsaturated fatty acids and polyunsaturated fatty acids were degraded faster in anoxic seawater than saturated fatty acids, and also differences in degradation rates of individual lipids (e.g., phytol) between the two species were observed (Harvey and Macko 1997). Our experiment with multiple species within several phytoplankton classes provides a more comprehensive test of how fatty acid composition may affect degradation rates of phytoplankton biomass than previous studies using only two species. With high variation in potential CO₂ and CH₄ production even within phytoplankton classes, it seems that anaerobic degradation processes were controlled by other factors than fatty acid composition. Also, our experiment included species with various cell-wall structures, for example, diatoms with silica frustules, chrysophyte with silica scales, green algae with cellulose cell-walls, and cyanobacteria with peptidoglycan cell-walls, and we conclude that cell-wall structure does not seem to determine potential CO₂ and CH₄ production from algal biomass in lake sediments.

High substrate C : N ratios may indicate lower lability for microbial degradation due to abundance of complex compounds such as polysaccharides or lignin and potential N limitation (Enríquez et al. 1993). The C : N ratios of algal biomass and sediment were rather low in our experiment (ca. 2–11 and 17.3, respectively), irrespective of the nutrient treatment, which likely explains the lack of effects in production potentials of CH₄ and CO₂. Furthermore, relatively high C : N ratio (\sim 48) of phytoplankton biomass did not result in lower potential CH₄ production in the study of West et al. (2015).

The accumulation of CH₄ during the anaerobic incubation typically follows a logistic curve after an initial lag-time; CH₄ production is initially limited by the colonization of the detritus particles by anaerobic microorganisms, followed by a substrate limitation at the end of a long incubation period (Segers and Kengen 1998; Kankaala et al. 2003; Vavilin et al. 2008). We detected a lag phase of ~ 15 d in CH₄ production in a preexperiment and, thus, the first measurement of the current study was conducted on day 16. Linear increase in CH₄ concentration continued until day 116 in most bottles, while CO₂ started to level off already after day 45. Since the bottles were only flushed in the beginning and not during the experiment, it is possible that volatile compounds, such as H₂S, accumulated in the bottles during the long incubation, and resulted in partial inhibition of CH₄ production (Karhadkar et al. 1987). However, we find significant inhibition unlikely since CH₄ production did not slow down during the incubation. Since the CH₄ production did not level off at the end of the incubation, we could not quantify the total amount of CH₄ produced from the algal biomass. The molar CH₄ : CO₂ ratio increased during the incubation, and was near the theoretical ratio (1:1) of complete OM degradation via methanogenesis in the end of the experiments (Conrad 2020).

Changes in CO₂ production rates during our experiment may show different phases of the biomass degradation process. Immediate increase in CO₂ when CH₄ was not produced shows active consumption of other electron acceptors (including oxygen) and fermentation that produces CO₂ and other substrates for methanogenesis. Very little CH4 was produced in controls, indicating that CO₂ production in controls was almost completely due to other processes than methanogenesis, for example, fermentation. This is also supported by the δ^{13} CO₂ of -24‰ in controls, which is close to the sediment value of -28%. CH₄ production, probably due to both acetoclastic and hydrogenotrophic methanogenesis, started after day 16, and CO₂ production continued, although at a slower rate. The CO₂ production leveled off at the end of the experiment while CH_4 production continued, and the $\delta^{13}C$ values of CH₄ (and CO₂) correlated strongly with CH₄ to CO₂ ratio at the end of the experiment. A single measurement of δ^{13} C values at the end of the experiment does not provide a complete picture of the dynamic degradation processes that take place during a long incubation, however, together with temporal trends in gas production they may give us some insights worth exploring. In our relatively long incubation experiment, the produced CO₂ was potentially recycled to CH₄ via hydrogenotrophic methanogenesis (Conrad 2020), based on the relatively high $\delta^{13}CO_2$ values and a decrease in CO₂ concentration at the end of the experiment. This corroborates the results from Ji et al. (2018) on rice field paddies, where hydrogenotrophic methanogenesis become relatively more important when labile OM became limiting during a long incubation. Thus, we suggest that C recycling is an important process in lake sediments as well. See Supporting

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Information for additional discussion on stable isotopes of CH_4 and CO_2 .

Conclusions

In natural conditions, algae and other substrates lead to enhanced CH₄ production during summer (Schulz and Conrad 1995), and similarly, algal additions were needed in order to start the CH₄ production in the current study. As noted above, nutrient limitation typically increases the lipid content of phytoplankton (Shifrin and Chisholm 1981; Rodolfi et al. 2008), and according to our first hypothesis and previous observations (e.g., West et al. 2015), such lipid rich substrate could have higher CH₄ production potential. This would then suggest that phytoplankton substrate in nutrient rich eutrophic lakes have lower potential, thereby reducing CH₄ production per unit substrate. However, the results presented here offer very little support for this hypothesis as only total fatty acid content was weakly related to CH₄ production while the lipid content (10-30%) of algae was not, meaning that the biomass from highly productive eutrophic lakes cannot be considered a lower quality substrate for CH₄ production.

Furthermore, there were no clear differences in potential gas production among phytoplankton classes, suggesting that fatty acid composition or cell-wall structure did not influence methanogenesis. The CH₄ emissions of lakes can be predicted from lake characteristics, such as area, depth, nutrient concentrations, and primary productivity (Bastviken et al. 2004; Deemer et al. 2016). The lack of clear effects of phytoplankton phylogenetic affiliation and lipid content on CH₄ production suggests that additional parameters related to phytoplankton community would not improve the estimates and are, thus, not needed. Our results on the effects of lipid content were in contrast with some previous studies (e.g., Zhao et al. 2014; West et al. 2015) and, thus, further studies with a wide range of biomass lipid content and of algal taxa that are common in lakes are still needed. Moreover, it is unclear how much lipid content of multispecies phytoplankton communities in lakes actually varies.

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Conflict of Interest

None declared.

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