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Phytoplankton lipid content influences freshwater lake methanogenesis

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SUMMARY

1. Rates of methanogenesis in freshwater sediments have been shown to increase with inputs of phytoplankton biomass. Although many studies have shown the importance of resource quality for decomposition, little is known of the importance of substrate quality on rates of methanogenesis.
2. Here, we studied the effects of lipid content and taxonomic affiliation of phytoplankton biomass on rates of methanogenesis in lake sediment slurries from five lakes differing in trophic status.
3. Substrate quantity had a positive effect on methanogenesis despite differing trophic status. Furthermore, we observed that phytoplankton biomass quality, in terms of lipid content, enhanced methanogenesis rates. However, rates of methanogenesis between lake sediments treated with *Scenedemus obliquus* or *Microcystis aeruginosa* did not differ when lipid content was held constant.
4. Phytoplankton lipid content has been shown to increase when nutrients are limiting, which may result in an increase in substrate quality for methanogenesis with eutrophication. However, our study revealed that responses of substrate quantity to nutrient enrichment likely outpace the effects of resource quality and may result in a net increase in CH_4 emissions from eutrophied lakes. Interestingly, the resource quality feedback may at least partially reduce the potential effect of eutrophication on lake methanogenesis.

Keywords: eutrophication, lakes, lipid, methane, phytoplankton

Introduction

Freshwater inland lake biotic processes are sensitive to changes in nutrient availability. Increased supply of mineral nutrients, often from anthropogenic sources, is known to increase phytoplankton productivity (Schindler, 1974; Hanson *et al.*, 2003; Smith, 2003). Enhanced nutrient availability can also alter the quality of phytoplankton biomass for consumers. For example, increases in phosphorus availability can lower the C:P ratio and alter essential fatty acid composition of phytoplankton biomass (Müller-Navarra *et al.*, 2004). Often these nutrient-associated shifts in phytoplankton biomass quantity and quality are mediated by shifts in phytoplankton community composition (Posch *et al.*, 2012). Subsequently, these nutrient-associated changes in phytoplankton resource composition and quality have been shown to strongly influence the growth rate of pelagic consumers (e.g. zooplankton; Elser, Hayakawa & Urabe, 2001; Park *et al.*, 2002).

In addition to pelagic consumers, sediment-dwelling decomposers are influenced by substrate quantity and quality (Suberkropp & Klug, 1976; Ward & Cummins, 1979; Hansen & Blackburn, 1992; Leroy & Marks, 2006; West *et al.* 2012). Recent work suggests that increases in substrate quantity, as occurs in a eutrophied system, can significantly increase methanogenesis rates in lake sediments (Schwarz, Eckert & Conrad, 2008; West *et al.*, 2012). However, despite demonstrable effects of substrate quality on decomposition processes (Hansen & Blackburn, 1992; Leroy & Marks, 2006), no studies have investigated the impact of phytoplankton biomass quality on rates of methanogenesis in lake sediments. The influence of substrate quality on methanogenesis is important to explore given its potential as a feedback on eutrophication-mediated increases in methanogenesis (Schwarz *et al.*, 2008; West *et al.*, 2012). Depending on whether phytoplankton quality, in terms of CH_4 yield, increases or decreases along a gradient of eutrophication, the response of methanogenesis to nutrient enrichment could be exac-

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erbated or ameliorated by shifts in phytoplankton biomass quality.

Although the importance of phytoplankton biomass quality for lake sediment methanogenesis has not been explored, previous work in engineered systems (bioreactors) suggests that taxonomic affiliation of phytoplankton biomass can influence CH_4 yields. One study observed a 50% reduction in CH_4 yields upon addition of cyanobacteria to an anaerobic bioreactor when compared to methanogenesis with a eukaryotic phytoplankton substrate (Zeng *et al*., 2010). The direct cause of reduced CH_4 yield from cyanobacterial biomass remains under-explored, but differences in biomass stoichiometry or macromolecular content are likely candidates. In fact, variation in lipid content of phytoplankton biomass has been shown to strongly alter methanogenesis rates in engineered systems (Zhao *et al*., 2014). Intuitively, the observed positive effect of lipid content of phytoplankton biomass on methanogenesis rates or CH_4 yield makes sense given the potential for rapid conversion of long-chain fatty acids to methanogenesis precursors (acetate, CO_2 and H_2) by fermenters and acetogens (Ferry, 1992; Thauer *et al*., 2008).

Phytoplankton intra- and inter-specific variation in lipid content spans a broad range (16–77%) of total cellular biomass (Harun *et al*., 2010). Interspecific variation is presumably driven by long-term adaptation. In contrast, intra-species lipid variation of eukaryotic phytoplankton has been linked to nutrient and light availability (Hu *et al*., 2008; Solovchenko *et al*., 2008; Rodolfi *et al*., 2009). For example, eukaryotic phytoplankton grown in nitrogen-deficient conditions often possess higher lipid concentrations (Piorreck, Baasch & Pohl, 1984; Griffiths & Harrison, 2009). Reduced nitrogen availability leads to a stress response, reducing cellular metabolism of many macromolecular components and often results in a shift from protein synthesis to lipid formation (Rosenberg *et al*., 2008; Rodolfi *et al*., 2009). Similar responses have been observed for phytoplankton grown with low availability of phosphorus (Lynn *et al*., 2000; Xin *et al*., 2010). When combined with the observation from engineered systems, that greater methane (CH_4) yield results from higher per cent lipid phytoplankton biomass, the potential for greater nutrient availability to diminish phytoplankton lipid content highlights a possible negative feedback for the effect of eutrophication on lake greenhouse gas production.

In this study, we used lake sediment treated with phytoplankton biomass of varied lipid content and taxonomic affiliation to evaluate the response of methanogenesis in five lakes differing in trophic status. We hypothesised that, regardless of lake trophic status, greater availability of labile phytoplankton substrate would enhance methanogenesis rates in freshwater lake sediment (quantity effect). We also hypothesised that increased lipid content of phytoplankton biomass (quality effect) would have a significant positive effect on methanogenesis in freshwater lake sediment, as has been previously observed in biofuel-related research (Collet *et al*., 2011). Finally, we evaluated the potential for taxonomic affiliation of phytoplankton biomass, specifically a switch from eukaryotic phytoplankton (*Scenedesmus obliquus*) to cyanobacteria (*Microcystis aeruginosa*), as is often observed during eutrophication, to modify rates of methanogenesis (Downing, Watson & McCauley, 2001).

Methods

Sampling sites

Five lakes (Bay, Brown, Crampton, Morris and North Gate) were sampled for sediment at the University of Notre Dame Environmental Research Center (UNDERC) near Land O′ Lakes, Wisconsin, U.S.A. (89.32′W, 42.13′N). These lakes varied considerably in size and spanned broad gradients of mixed layer total phosphorus, total nitrogen, chlorophyll, dissolved organic carbon and sediment organic matter (Table 1).

Water chemistry

At each lake, water was collected from the upper mixed layer for analysis of water chemistry. Total phosphorus (following persulfate digestion) was measured using a

Table 1 Summary table of lake characteristics, including lake area, max depth, sediment pH, sediment organic matter (SOM), chlorophyll (Chl), dissolved organic carbon (DOC), total phosphorus (TP) and total nitrogen (TN)

Lake	Area (ha)	Max depth (m)	Sediment pH	SOM (%)	Chl (μg L^{-1})	DOC (mg L^{-1})	TP (μg L^{1})	TN (μg L^{-1})
Bay	69.7	12.2	7	15.5	4.4	6.5	22.8	415
Brown	29.6	4.9	8.3	20.4	40.2	6.6	86.9	892
Crampton	25.9	18.5	5.9	25.2	4	4.5	11.1	519
Morris	5.9	6.7	7.8	26	7.2	22.6	36.2	849
North Gate	0.2	8	4.7	28.3	32.3	23.4	17.6	676

colorimetric assay (Menzel & Corwin, 1965), chlorophyll a was analysed using methanol extraction and fluorometry (Welschmeyer, 1994) and dissolved organic carbon was analysed using a Shimadzu TOC-V total organic carbon analyser (Shimadzu Scientific Instruments, Kyoto, Japan).

Phytoplankton growth conditions

Phytoplankton strains of *M. aeruginosa* and *S. obliquus* were grown in Bold's Basal Medium (BBM). To obtain eukaryotic phytoplankton biomass with two distinct lipid concentrations, we grew *S. obliquus* with two nitrogen concentrations: 6.7 and 2.2 mg N L^{-1}, whilst *M. aeruginosa* was only grown at 6.7 mg N L^{-1}. All phytoplankton were grown under the same light source and bubbled with atmospheric carbon dioxide concentrations to prevent CO_2 limitation, until growth reached stationary phase. Previous work has demonstrated that by reducing nitrogen availability to a wide range of eukaryotic phytoplankton species, including *S. obliquus*, lipid anabolism increases and phytoplankton store greater quantities of carbon as lipid (Piorreck *et al.*, 1984; Griffiths & Harrison, 2009; Dean *et al.*, 2010; Liu *et al.*, 2012; Adams *et al.*, 2013; Adams & Bugbee, 2014). Although we chose to use nitrogen availability to alter lipid content, previous work suggests we also could have accomplished this by altering phosphorus availability (Lynn *et al.*, 2000; Xin *et al.*, 2010).

Phytoplankton lipid content and stoichiometry

To compare lipid content of *M. aeruginosa* and *S. obliquus* grown under different nitrogen conditions, we extracted and analysed lipids from the phytoplankton biomass using methods from Heissenberger, Watzke & Kainz (2010). Freeze-dried phytoplankton samples (~200 mg wet weight) were stored overnight in chloroform (2 mL) at −80 °C. Sodium chloride (0.8 mL) was added to each sample, and the samples were subsequently homogenised with glass beads. Addition of a 2 : 1 chloroform: methanol solution (1 mL) and centrifugation were used to extract lipids from each homogenised sample. The organic lipid-containing layer was extracted with subsequent washes with chloroform and evaporated with N_2 down to 0.5 mL. An aliquot (0.1 mL) of extracted lipids was dried and weighed to obtain total lipid mass per sample. Carbon and nitrogen content of extracted lipids and whole freeze-dried phytoplankton biomass were analysed using an elemental analyser (ECS 4010; Costech Analytical Technologies Inc., Valencia, CA, U.S.A.). To quantify the phosphorus content of phytoplankton lipids, we digested the phytoplankton lipids with perchloric acid (Hatzakis *et al.*, 2008). Phosphorus content of phytoplankton biomass and perchloric acid digested lipids was measured using a colorimetric assay following persulfate digestion (Menzel & Corwin, 1965).

Experimental design

Between 6 June, 2014 and 19 June, 2014, sediment was collected using an Ekman sampler (Wildlife Supply Company, Yulee, FL, U.S.A.) at the deepest location in each of the five lakes. Upon returning to the laboratory, we added 50 mL of hypolimnion water, obtained from 0.5 m above the sediment surface with a Van Dorn sampler, and 50 mL of sediment to a 300-mL serum bottle. Four treatments, with quadruplicate slurries (total of 16 slurries per lake), were run for each of the five lakes: no algal addition, addition of 20 mg of dried high-lipid *S. obliquus*, addition of 20 mg of dried low-lipid *S. obliquus* and addition of 20 mg of dried *M. aeruginosa*. The serum bottles were capped with rubber septa and aluminium crimp seals. The remaining 200 mL headspace was purged with N_2 gas to insure anoxic conditions. The slurries were incubated at approximately 4 °C (near *in situ* sediment temperature) in the dark for 28 days. Every 5 days, starting on the third day, a 10-mL gas sample was extracted from each slurry headspace, injected into a GC vial for CH_4 quantification and 10 mL of N_2 was added back to the slurry bottles to maintain atmospheric pressure. After accounting for headspace dilution due to sampling, methanogenesis rates were inferred from the slope of linear regressions fit to the time courses of CH_4 concentrations.

Gas chromatography

All CH_4 samples were 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 m, 0.32 mm and 3.0 μm, respectively (Agilent Technologies, Santa Clara, CA, U.S.A.). Samples were analysed in split mode (5 : 1 ratio), and the inlet, oven and FID temperatures were 185, 30 and 250 °C, respectively. The carrier gas total flow and split flow were 19.1 and 13.5 mL min^{-1}, respectively. The air, hydrogen and helium flow rates passing through the FID were 300, 40 and 40 mL min^{-1}, respectively.

Sediment organic matter

Replicate subsamples of sediment from each Ekman grab were dried overnight in a 60 °C oven and the dry

weight was recorded. Subsequently, organic matter in the dry sediment was combusted at 550 °C for four hours, and the sediment was reweighed. Sediment organic matter was estimated as a per cent of sediment material lost on ignition. Organic matter content varied little across lakes (Table 1).

Statistical methods

Based upon Shapiro–Wilk tests, the residuals of the observed rates of methanogenesis were not normally distributed. As a result, we square-root transformed the data and performed a two-way analysis of variance (ANOVA) to test our hypothesised effects of phytoplankton quantity, quality or taxonomic affiliation and a lake effect on methanogenesis rates. Tukey's honestly significant difference (HSD) test with a 95% family-wise confidence level was used for *post hoc* comparisons. All statistical analyses were carried out in the base R statistical package (R Core Team, 2014).

Results

Phytoplankton and lipid stoichiometry

Scenedesmus obliquus grown under 2.2 and 6.7 mg N L^{-1} contained 0.28 and 0.14 mg lipid per mg dry weight, respectively. *M. aeruginosa* grown in 6.7 mg N L^{-1} BBM, yielded biomass with 0.16 mg lipid per mg dry weight. Mean carbon content of *M. aeruginosa*, low-lipid *S. obliquus* and high-lipid *S. obliquus* was 63.3, 61.5 and 69.5%, respectively. Phytoplankton biomass nitrogen content was reduced in high-lipid *S. obliquus* (grown in low-nitrogen media) relative to *M. aeruginosa* and low-lipid *S. obliquus*, but C:P ratios were comparable across growth conditions and species (Table 2). Carbon content of extracted lipids varied between *M. aeruginosa* and *S. obliquus* (0.64 versus 0.77 mg C mg lipid $^{-1}$), but did not differ between low- and high-lipid *S. obliquus*. The C:N:P ratio of lipids varied more substantially across species and growth conditions than stoichiometric ratios of total biomass (Table 2).

Phytoplankton biomass quantity effects

For all five lakes, sediment slurries with phytoplankton substrate added had significantly greater rates of methanogenesis (two-way ANOVA; $P < 0.001$, Fig. 1). We also observed significant differences in the magnitude of response to phytoplankton biomass additions across lakes (two-way ANOVA, lake by treatment interaction term, $P < 0.001$, Fig. 2). However, we were unable to identify any lake characteristics (Table 1), such as mixed layer chlorophyll, DOC, TP, sediment pH or sediment organic matter, that predicted the magnitude of response to phytoplankton additions across lakes.

Table 2 Stoichiometry of biomass and lipid content of high- and low-lipid *Scenedesmus obliquus* and *Microcystis aeruginosa*

Phytoplankton species	Phytoplankton C:N:P	Lipid C:N:P
High-lipid *S. obliquus*	158 : 3.8 : 1	360 : 2 : 1
Low-lipid *S. obliquus*	149 : 13 : 1	207 : 6.2 : 1
M. aeruginosa	133 : 13 : 1	65.3 : 4.7 : 1

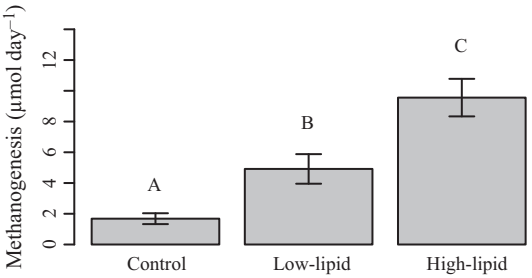

Fig. 1 Mean methanogenesis rates of sediment slurries from control, low-lipid and high-lipid phytoplankton treatments. Error bars indicate one standard error. Letters above bars indicate significance of Tukey's HSD *post hoc* tests ($P < 0.001$ for all comparisons).

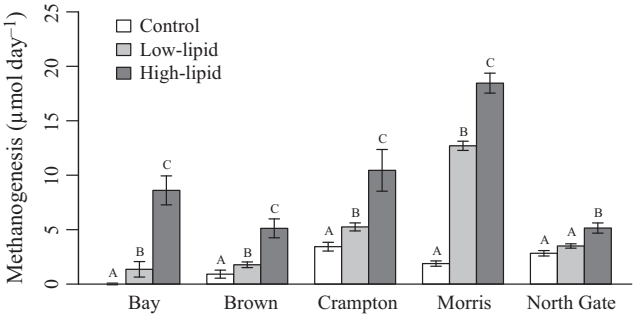

Fig. 2 Individual lake mean methanogenesis rates of sediment slurries with added high-lipid *Scenedesmus obliquus* biomass, low-lipid *S. obliquus* biomass and control. Error bars indicate one standard error. Letters above each treatment signify statistically significant differences within a single lake, as assessed with Tukey's HSD *post hoc* tests.

Phytoplankton biomass quality effects

To compare how phytoplankton substrate quality may influence methanogenesis, we compared methanogenesis rates in slurries with *S. obliquus* of high (0.28 mg lipid mg dw^{-1}) and low-lipid content

(0.14 mg lipid mg dw^{-1}). Higher rates of methanogenesis were found in all lake sediments with high-lipid *S. obliquus* additions compared to low-lipid *S. obliquus* additions (Tukey's HSD *post hoc*, $P < 0.001$). Across all lakes, the high-lipid *S. obliquus* treatments increased methanogenesis by, on average, 1.95 times compared to low-lipid *S. obliquus* treatments (Fig. 1).

Effect of phytoplankton biomass taxonomic affiliation

By comparing methanogenesis rates from sediment slurries treated with *M. aeruginosa* and *S. obliquus* grown under the same conditions and possessing similar lipid content, we were able to quantify the effect of phytoplankton taxonomy, at least for two taxa, without confounding lipid content effects. We did not observe a significant difference between methanogenesis rates in low-lipid *S. obliquus* and *M. aeruginosa* treatments (Tukey's HSD *post hoc*; $P = 0.73$, Fig. 3), but we observed similar increased responses relative to controls (Tukey's HSD *post hoc*; $P < 0.001$). The only lake that displayed significant differences between *S. obliquus* and *M. aeruginosa* was Morris Lake (Tukey's HSD *post hoc*; $P < 0.001$).

Discussion

The quantity, quality (e.g. lipid content) and taxonomic affiliation of phytoplankton biomass have all been shown to enhance methanogenesis in natural or engineered systems (Collet *et al.*, 2011; West *et al.*, 2012). To explicitly consider each of these potential controls on lake sediment methanogenesis, we conducted a series of laboratory-scale incubation experiments. To modify resource quality, we exploited the propensity for *S. obliquus* to increase its lipid content with reduced nitrogen availability to generate phytoplankton biomass of differing lipid content. As expected, *S. obliquus* cultures grown with two different nitrogen concentrations differed in lipid content (14 and 28% of biomass for high- and low-nitrogen concentrations, respectively). The high-lipid *S. obliquus* had 0.08 mg C $(mg\ dw)^{-1}$ more than the low-lipid *S. obliquus*, but 0.11 mg lipid-C $(mg\ dw)^{-1}$ more than the low-lipid *S. obliquus*. The fact that low-nitrogen, high-lipid *S. obliquus* showed greater gains in lipid carbon than total carbon suggests some reallocation of intracellular carbon, as has been observed previously, but it appears much of the lipid gains were due to increases in per cell carbon content.

Methanogenesis was enhanced in each lake sediment when treated with additional phytoplankton biomass indicating that lake methanogenesis was substrate limited. The degree of substrate limitation was expected to decrease with an increase in lake primary productivity, but we observed no evidence to support this conjecture. Sediment slurries from each lake responded strongly to treatments of additional phytoplankton biomass (response ratios of 1.24–6.72; not calculated for Bay lake, as control-slurry methanogenesis rates were zero), but the magnitude of response was not related to any of lake characteristics tested here, including common proxies for lake productivity. Consistent results across five lakes suggest that previous findings, indicating that phytoplankton is a labile substrate for methanogenesis, may be more general than previously thought, and even sediments of productive lakes or those receiving large amounts of less labile terrestrial carbon (Sobek *et al.*, 2009; West *et al.*, 2012) are limited by phytoplankton substrate availability (Schwarz *et al.*, 2008; West *et al.*, 2012).

Our experiments also revealed the potential for phytoplankton biomass lipid content to influence rates of methanogenesis, with higher lipid content yielding greater rates. The molar yield of CH_4 across our 25-day incubations ranged from 0.08 for the low-lipid to 0.17 for the high-lipid *S. obliquus* treatment. However, yields of CH_4 per mg lipid were similar across the treatments (1.47 μmol CH_4 mg lipid-C^{-1} d^{-1} for low-lipid and 1.83 μmol CH_4 mg lipid-C^{-1} d^{-1} for high-lipid), resulting in a doubling of methanogenesis in response to the doubling of *S. obliquus* lipid carbon content (10.7–21.6% lipid carbon). Since CH_4 yields from phytoplankton carbon were 2.13 times higher between high and low-lipid treatments, and we observed a similar CH_4 yield from lipid in both treatments (1.47 and 1.83 μmol CH_4 mg lipid-C^{-1} d^{-1} for low- and high-lipid *S. obliquus*,

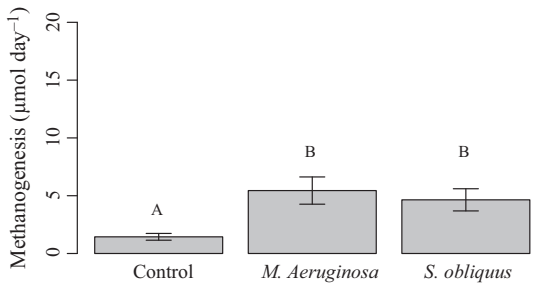

Fig. 3 Mean methanogenesis rates of sediment slurries receiving no algal additions, *Microcystis aeruginosa* and low-lipid *Scenedesmus obliquus* biomass across all lakes. The *M. aeruginosa* and *S. obliquus* biomass contained similar lipid content of 0.16 and 0.14 mg lipid mg^{-1} dry weight, respectively. Error bars indicate one standard error, and letters indicate significant *post hoc* differences.

respectively), we can conclude that changes in phytoplankton macromolecular composition, that is lipid content, and not just total algal C availability, are an important regulator of fermentation and subsequent methanogenesis rates in freshwater lake sediment. Our data are consistent with previous work in engineered systems, suggesting that phytoplankton lipid content is the predominant regulator of methanogenesis (Park & Li, 2012).

Many previous studies have highlighted the importance of resource stoichiometry for consumer activity and growth, including that of decomposers (Elser *et al.*, 2001; Park *et al.*, 2002; Posch *et al.*, 2012). In these cases, substrate with reduced C:N or C:P are usually of greater resource quality (Park *et al.*, 2002; Posch *et al.*, 2012). Our results indicate that phytoplankton biomass stoichiometry was unlikely to influence rates of methanogenesis. Because low-lipid *S. obliquus* was grown in high-nitrogen medium its C:N ratio in biomass and lipids were lower than that of high-lipid *S. obliquus*, but this did not result in greater yields of CH_4. Rather high-lipid *S. obliquus* (relatively high C:N) generated the most CH_4, suggesting lipid content and not stoichiometry is the key regulator of sediment methanogenesis.

Previous work has shown taxonomic differences in macromolecular investment amongst phytoplankton, including variation in lipid, protein and carbohydrate content (Piorreck *et al.*, 1984; Griffiths & Harrison, 2009; Wiley, Campbell & McKuin, 2011). Although we have demonstrated the strong role cellular lipid content can play, we also evaluated the potential for additional taxonomic differences in CH_4 yield when lipid content is held constant. Exhaustive comparison of a large number of taxonomic groups was beyond the scope of our experiment; therefore, we chose to compare CH_4 yield in lake sediments treated with *S. obliquus* or *M. aeruginosa*, as phytoplankton community composition often becomes dominated by cyanobacteria as a lake is eutrophied (Downing *et al.*, 2001). With similar lipid content between *S. obliquus* and *M. aeruginosa*, any effects of potential taxonomic differences beyond lipid content on methanogenesis were negligible. Lack of a taxonomic response to additions of *S. obliquus* and *M. aeruginosa* with similar lipid content further supports the importance of lipid content as a metric of substrate quality for fermentation and subsequent methanogenesis. More work, with a larger and more diverse set of phytoplankton, is needed to fully evaluate taxonomic effects on CH_4 yields. However, homogenisation of macromolecular compositional differences by fermenter-mediated generation of the limited number of substrates (H_2, CO_2, and acetate) utilised by methanogens likely diminishes expectations for taxonomic substrate effects (Thauer *et al.*, 2008).

Although caution is advised when extrapolating findings from microcosms to whole ecosystems (Carpenter, 1996), the consistent and strong response of lake sediment methanogenesis rates to shifts in the quantity and quality of phytoplankton biomass supply indicates a potential link between lake eutrophication and methanogenesis. Interestingly, the elevated nutrient supply that leads to cultural eutrophication of lake ecosystems would be expected to increase the quantity, but reduce the quality, of phytoplankton biomass settling to anoxic lake sediments (Fig. 4; Paerl *et al.*, 2001, 2011; Adams *et al.*, 2013). Accordingly, reduction in lipid content of algal cells (i.e. quality) with greater nutrient availability (Paerl *et al.*, 2001; Griffiths *et al.*, 2009; Paerl *et al.*, 2011; Adams *et al.*, 2013) could act to diminish the impact of elevated primary productivity and phytoplankton biomass sedimentation resulting from greater nutrient supply. The relative importance of the quantity and quality effects on methanogenesis and magnitude of change in these variables along a lake trophic status will determine the net effect of eutrophication on lake methanogenesis.

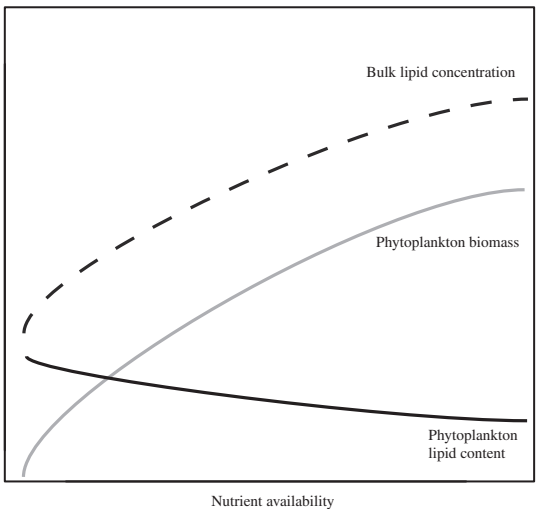

Fig. 4 A conceptual figure depicting hypothesised relationships between nutrient availability (N and/or P), phytoplankton biomass, lipid content and bulk lipid concentration. Phytoplankton biomass and bulk lipid concentration relationships were based on the literature review averages of phytoplankton biomass and bulk lipid concentration conducted by Griffiths & Harrison (2009) and data from Piorreck *et al.* (1984). The negative relationship between nutrient availability and lipid content was demonstrated in Piorreck *et al.* (1984). The positive, nonlinear relationship between phytoplankton biomass in lakes and nutrient availability is well accepted (Dillon & Rigler, 1974; Filstrup *et al.*, 2014).

Our experiments and previous work have shown the effects of phytoplankton biomass quantity and quality on methanogenesis along a trophic gradient. In our study, response ratios for quantity and quality effects were similar (2.85 ± 2.6 and 1.95 ± 0.67, respectively), although this comparison is confounded by the relative strength of our manipulations of resource quantity (20 mg dry biomass) and quality (increase of lipid content from 14 to 28% of dry biomass). In addition, eutrophication-driven increases in phytoplankton biomass span approximately two orders of magnitude, with oligotrophic lakes containing *c.* 0.01 mg phytoplankton C L^{-1} compared to *c.* 2 mg phytoplankton C L^{-1} in hyper-eutrophic systems (Hessen *et al.*, 2003). Per cell lipid content is obviously constrained between 0 and 100%, but field and laboratory observations suggest that the range is between 16 and 77% lipid content (Harun *et al.*, 2010). The dynamic range of these two factors and the relative strengths of their effects on methanogenesis suggest a net increase in bulk lipid concentration (lipid per cell $\times$ biomass) along a trophic gradient of freshwater lakes (Fig. 4). However, a loss of phytoplankton lipid content meaningfully reduces our expectations for methanogenesis along a gradient of lake trophic status.

While, current data and scenarios outlined in this study illustrate that increases in phytoplankton biomass are correlated with increased bulk lipid concentration, data relating per cell or total lipid content to nutrient availability from natural systems are limited. In addition, laboratory experiments suggest that light availability, which would also interact with phytoplankton biomass via self-shading, can modify per cell lipid content of phytoplankton as well (Hubble & Harper, 2001; Solovchenko *et al.*, 2008). For example, decreased light availability can result in a threefold reduction of lipid productivity by phytoplankton in engineered systems (Ho, Chen & Chang, 2012). Therefore, at a lake scale, understanding how nutrient supply, lake water colour and phytoplankton self-shading interact to modify bulk lipid content, which appears to strongly control methanogenesis rates, could be important for predicting freshwater lake CH_4 dynamics.

This study was the first to demonstrate that phytoplankton lipids are an important regulator of sediment methanogenesis in freshwater lakes. Furthermore, our study revealed that the effect of taxonomic differences in phytoplankton substrate on methanogenesis in lake sediments is likely negligible in comparison to the effect of biomass quantity and per cell lipid content. Along an increasing gradient of lake trophic status, biomass appears to most strongly influence bulk lipid concentrations, which is a key regulator of freshwater lake methanogenesis rates, but shifts in per cell lipid content partially counteract the impact of increased biomass. As a result, global trends in cultural eutrophication and associated increases in bulk lipid concentration are likely driving increased methanogenesis rates and the potential for increased CH_4 emissions from freshwater lakes.

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