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Global co-occurrence of methanogenic archaea and methanotrophic bacteria in *Microcystis* aggregates

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Summary

Global warming and eutrophication contribute to the worldwide increase in cyanobacterial blooms, and the level of cyanobacterial biomass is strongly associated with rises in methane emissions from surface lake waters. Hence, methane-metabolizing microorganisms may be important for modulating carbon flow in cyanobacterial blooms. Here, we surveyed methanogenic and methanotrophic communities associated with floating *Microcystis* aggregates in 10 lakes spanning four continents, through sequencing of 16S rRNA and functional marker genes. Methanogenic archaea (mainly *Methanoregula* and *Methanosaeta*) were detectable in 5 of the 10 lakes and constituted the majority (~50%–90%) of the archaeal community in these lakes. Three of the 10 lakes contained relatively more abundant methanotrophs than the other seven lakes, with the methanotrophic genera *Methyloparacoccus*, *Crenothrix*, and an uncultured species related to *Methylobacter* dominating and nearly exclusively found in each of those three lakes. These three are among the five lakes in which methanogens were observed. Operational taxonomic unit (OTU) richness and abundance of methanotrophs were strongly positively correlated with those of methanogens, suggesting that their activities may be coupled. These *Microcystis*-aggregate-associated methanotrophs may be responsible for a hitherto overlooked sink for methane in surface freshwaters, and their co-occurrence with methanogens sheds light on the methane cycle in cyanobacterial aggregates.

Introduction

Cyanobacterial harmful algal blooms (cyanoHABs) in freshwater lakes have reached unprecedented levels in recent years. This increase is a global ecological issue, mainly due to depletion of oxygen from surface waters and release of toxins, which can severely impact ecosystem function and human health (Huisman *et al*., 2018). The increased frequency of cyanoHABs is in general linked to excessive nutrient inputs and rising global temperatures (Paerl and Otten, 2013; Mantzouki *et al*., 2018). Most

cyanoHABs are composed of planktonic aggregates, usually containing a dominant cyanobacterial species as a primary producer accompanied by diverse heterotrophic bacteria, which may contribute to the proliferation and persistence of cyanobacterial blooms (e.g., through recycling of nutrients, generation of CO_2, and detoxification of oxygen radicals) (Dziallas and Grossart, 2011; Dziallas and Grossart, 2012; Paerl and Otten, 2013; Cook *et al*., 2020). The community composition of the associated heterotrophic bacteria can vary with size of aggregates (Cai *et al*., 2014), while anoxic microniches important for anaerobes are more likely to occur in larger aggregates (Klawonn *et al*., 2015) and scums.

Although we are beginning to get a clearer picture of the nitrogen cycle within cyanobacterial aggregates (Klawonn *et al*., 2015), the role of methane cycling microbes (methanogens and methanotrophs) has been addressed to a lesser degree, but it is clearly very important, as enhanced eutrophication of freshwater lakes promotes growth of photoautotrophs and methane emissions from surface waters of lakes have been positively linked to methane production by Cyanobacteria and other algae (Grossart *et al*., 2011; Bogard *et al*., 2014; Bizic *et al*., 2020; Günthel *et al*., 2020). Increased eutrophication has been predicted to result in a substantial increase (30%–90%) in methane emissions to the atmosphere over the next century (Beaulieu *et al*., 2019), but the linkage to cyanobacterial blooms has gained little scientific attention.

Microbial methane production in lakes was thought to primarily occur in anoxic sediments, from where updiffused methane would be depleted by aerobic methane oxidation in the surface layers of sediment or in the water column (Bastviken *et al*., 2008). Yet, there have been several reports showing the presence and activity of methanogenic archaea associated with photoautotrophs in oxic lake waters (Grossart *et al*., 2011; Bogard *et al*., 2014), as well as the occurrence of methanogens in isolated *Microcystis* aggregates (Batista *et al*., 2019). Their presence is likely due to temporary anoxic microniches within planktonic aggregates (Paerl and Pinckney, 1996; Dziallas and Grossart, 2012; Klawonn *et al*., 2015).

Methane oxidation can be rapid in the presence of oxygen, e.g., during seasonal lake turnover (Mayr *et al*., 2020). In addition, methane oxidation coupled to oxygenic photosynthesis, allowing methane oxidation and subsequent symbiotic carbon transfer to the phototroph, has been documented in the anoxic hypolimnion of lakes and in peatlands (Raghoebarsing *et al*., 2005; Milucka *et al*., 2015). Until now, there are no studies on the occurrence and microscale distribution of methanotrophs within cyanobacterial aggregates. Given previous observations of co-occurring aerobic and anaerobic microbial nitrogen cycling processes across steep oxygen gradients within cyanobacterial aggregates (Klawonn *et al*., 2015), it is likely that methanotrophy is indeed coupled with archaeal methanogenesis within *Microcystis* aggregates.

At circumneutral pH, aerobic oxidation of methane is carried out by methane-oxidizing bacteria (MOB) belonging to Alphaproteobacteria and Gammaproteobacteria (Hanson and Hanson, 1996). The *pmoA* gene encoding a subunit of the particulate methane monooxygenase is commonly used as a biomarker to detect MOB, as phylogenies derived from it are largely congruent with those of the 16S rRNA gene (Knief, 2015). In addition, methanogenic members within the archaeal phylum Euryarchaeota universally use methyl-coenzyme M reductase, and the associated *mcrA* gene is typically used as functional biomarker for investigating methanogenic archaea communities (Thauer *et al*., 2008).

The goals of this study were (i) to investigate the distribution of methane cycling microbes associated with cyanobacterial blooms and (ii) to investigate their relationships based on abundances and richness of marker genes to predict whether activities of methanogens and methanotrophs are linked to each other. We focused primarily on *Microcystis* blooms in freshwater lakes and collected aggregate samples from 10 lakes across four continents (Supporting Information Fig. S1).

Results

Cyanobacterial reads were predominant in each aggregate sample (Supporting Information Fig. S2), most of which (>82% of Cyanobacteria) were further classified to the *Microcystis* genus, confirming that *Microcystis* was the major bloom-forming component in all aggregate samples.

Molecular characterization of methanogenic archaea in cyanobacterial bloom aggregates

To explore the potential for biotic methane production within the cyanobacterial aggregates, the presence of methanogenic archaea in aggregate DNA extracts was examined by generating archaeal 16S rRNA gene libraries. Although archaeal reads were nearly absent from 5 of the 10 sampled lakes, a total of 301 OTUs from 48 135 high-quality archaeal reads were successfully retrieved from the remaining five lakes (Fig. 1A). These latter five included the group of three lakes with a high proportion of methanotrophs (see below, i.e., high MOB group). Methanogens belonging to six orders of the phylum Euryarchaeota, together comprised 50%–90% of the total archaeal community across these lakes (Fig. 1A). In addition, the other major archaeal lineages

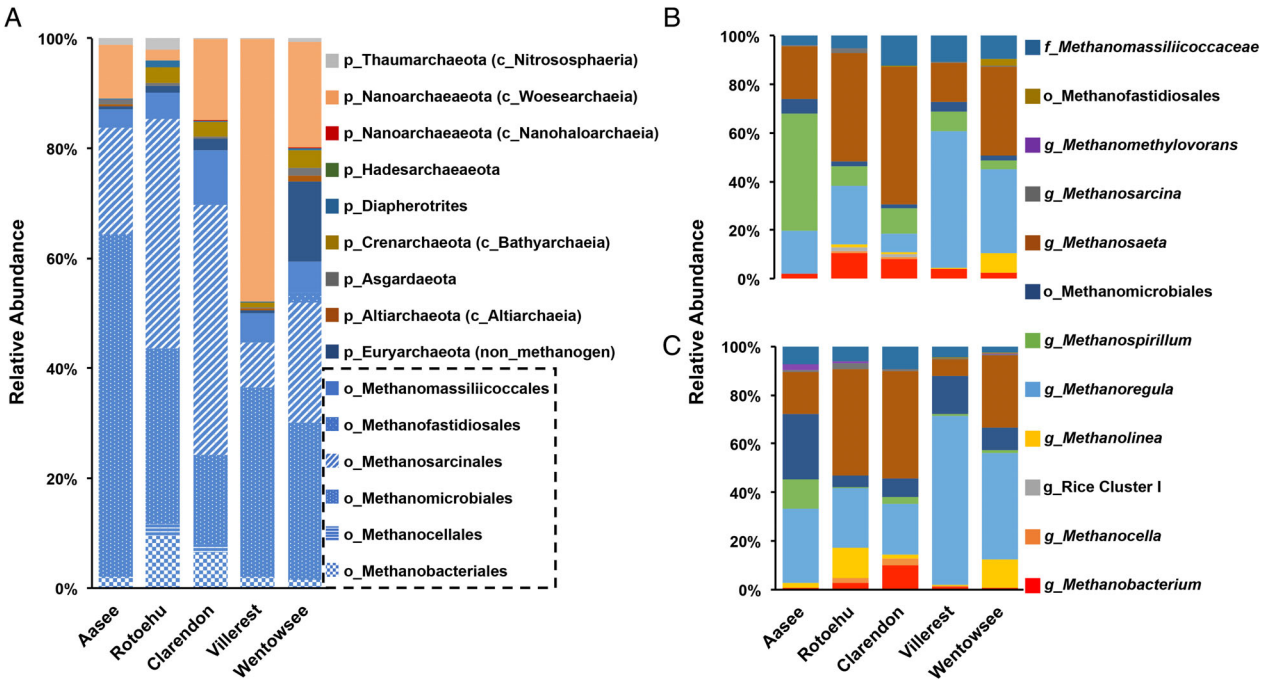

Fig. 1. Distribution of methanogenic lineages and other archaeal phyla across five lakes. A. Composition of the archaeal community based on archaeal 16S rRNA gene sequencing. The blue colour lineages in the dashed line represent the six methanogenic orders. The composition of the methanogenic community at the genus level is shown in B based on archaeal 16S rRNA gene sequencing and C based on *mcrA* gene sequencing. The following taxonomic levels are used: p – phylum, c – class, o – order, f – family, g – genus. [Color figure can be viewed at wileyonlinelibrary.com]

were represented by Woesearchaeia and Bathyarchaeia, along with ~0.63%–3.6% of sequences affiliated with five other phyla (Fig. 1A). Within the six methanogenic orders, members of the two genera *Methanosaeta* and *Methanoregula* dominated the methanogenic communities (together accounting for 39.1%–72.4% of reads) across the five lakes (Fig. 1B).

Additionally, we targeted the *mcrA* gene for further proof of their presence and for phylogenetic analysis of methanogens. The *mcrA* genes could not be amplified via PCR in the lakes that did not yield any archaeal 16S gene products. With identical PCR conditions and sequencing runs, the other five lakes yielded the correct sized amplicons with the number of identified *mcrA* reads ranging from 9936 to 31 851 per lake. Given the consistent distribution pattern observed with the two types of genes, we confirmed that methanogens occurred in 5 of the 10 lakes and were most likely to be either absent or present in negligible numbers in the others. Phylogenetic analysis of representative sequences of the 41 *mcrA* OTUs showed that all fell within the same orders of Euryarchaeota as determined by 16S rRNA gene analysis. The majority of the OTUs determined by *mcrA* analysis were similarly closely related to the methanogenic genera identified by 16S gene analysis (Figs. 1 and 2). The composition of methanogenic communities among the lakes uncovered by *mcrA* analysis (Fig. 1C) also confirms the dominance of *Methanosaeta* and *Methanoregula* among the detected methanogens. As well, six OTUs within the order Methanomicrobiales robustly form monophyletic clades with environmental clones, supported by high bootstrap values (Fig. 2; labelled OTU17, 27,41,56,62,78). OTU17 (the fourth most abundant *mcrA* OTU representing 10.6% of total *mcrA* reads) was broadly distributed among lake samples and clustered with a clone (97.16% similarity) from Lake Dianchi which is known to have persistent blooms (Yang *et al*., 2016). Using a summary of the range of substrates utilized by each specific methanogenic lineage (Supporting Information Table S1) (Evans *et al*., 2019), we observed an abundance of methanogens with the potential to obligately perform hydrogenotrophic or acetoclastic methanogenesis, constituting 87.2% to 96.2% of total methanogens across the five lakes (Supporting Information Fig. S3), suggesting that acetate and H_2 constitute their main energy sources, obtained through a direct interaction with fermentative bacteria, rather than being active in degradation of methylated substrates.

As the same lineages of dominant methanogens were observed in previous studies (Grossart *et al*., 2011, Batista *et al*., 2019), this raises the question of whether cyanobacterial aggregates share a similar methanogenic community. The dissimilarity test (Adonis) based on weighted UniFrac distance of *mcrA* genes showed significant

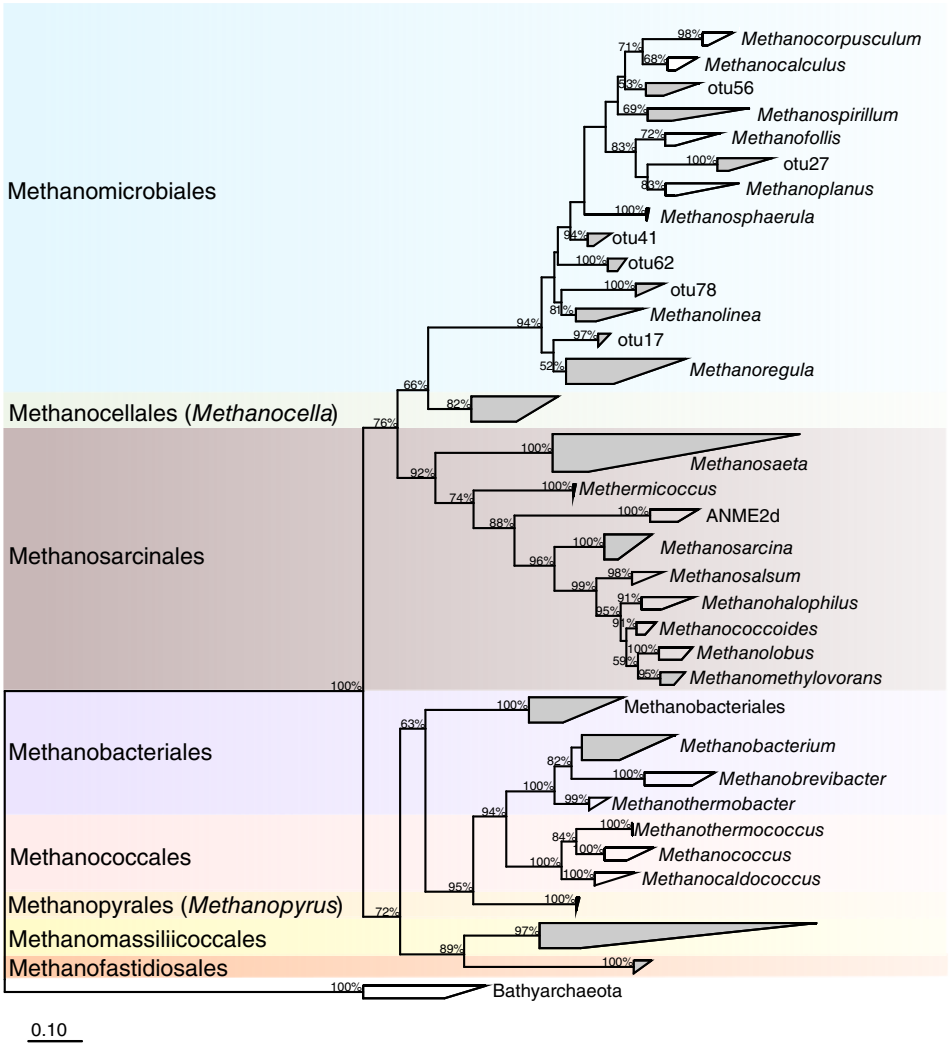

Fig. 2. Maximum likelihood phylogenetic tree based on aligned partial amino-acid sequences of the *mcrA* gene. The tree was calculated with RAxML 8.2.12 using PROTMIX-JTT evolutionary model and using the rapid bootstrap algorithm with 100 iterations. Bootstrap values above 50% are displayed next to the nodes. Shaded clades contain the OTU sequences that were detected in this study. The clades were clustered into eight methanogenic orders as indicated by background colour. The *mcrA* containing lineage from the phylum Bathyarchaeota was used as outgroup. [Color figure can be viewed at wileyonlinelibrary.com]

differences in the phylogenetic structure of methanogenic communities (at the OTU level) among five lakes ($R^2 = 0.85$, $P = 0.001$) (Supporting Information Fig. S5c). Correspondingly, a principal coordinates analysis (PCoA) plot demonstrated that methanogenic communities (determined with *mcrA*) are clearly divided into three groups (1-Lakes Clarendon and Rotoehu; 2-Wentowsee; 3-Lac de Villerest and Aasee), based on their clear separation along the first axis of the plot (Supporting Information Fig. S5c). This pattern was consistent with data from procrustes analysis using the archaeal 16S gene dataset ($m^2 = 0.286$, $P < 0.001$) (Supporting Information Fig. S4).

Molecular characterization of MOB community in cyanobacterial bloom aggregates

After removal of 16S cyanobacterial sequences, the remainder were used to describe the MOB community with 5831 MOB sequences clustered into 35 OTUs (Supporting Information Fig. S6). Among the 10 lakes, type II MOB belonging to *Methylocystis* were ubiquitous and present at similar levels across all lakes, whereas some specific type I MOB genera were nearly exclusively found in one lake and not in the others (Fig. 3A). Specifically, *Methyloparacoccus* and one of the eight OTUs associated with unclassified *Methylomonaceae* co-dominated the MOB community in Lake Clarendon, constituting 1% and 0.74% of the non-cyanobacterial sequences respectively (Fig. 3A). The latter unclassified OTU was found to be most similar (99.3%) to a strain of the very recently defined novel genus *Methylicorpusculum* from an oil sands tailings pond (Saidi-Mehrabad *et al*., 2020). In Lac de Villerest, the genus *Crenothrix*, with remarkably high relative abundance, up to 2.38%, outnumbered the other genera (Fig. 3A). The lineage pLW-20 (Nercessian *et al*., 2005) accounted for over 1.45% of non-cyanobacterial sequences in Wentowsee (Fig. 3A).

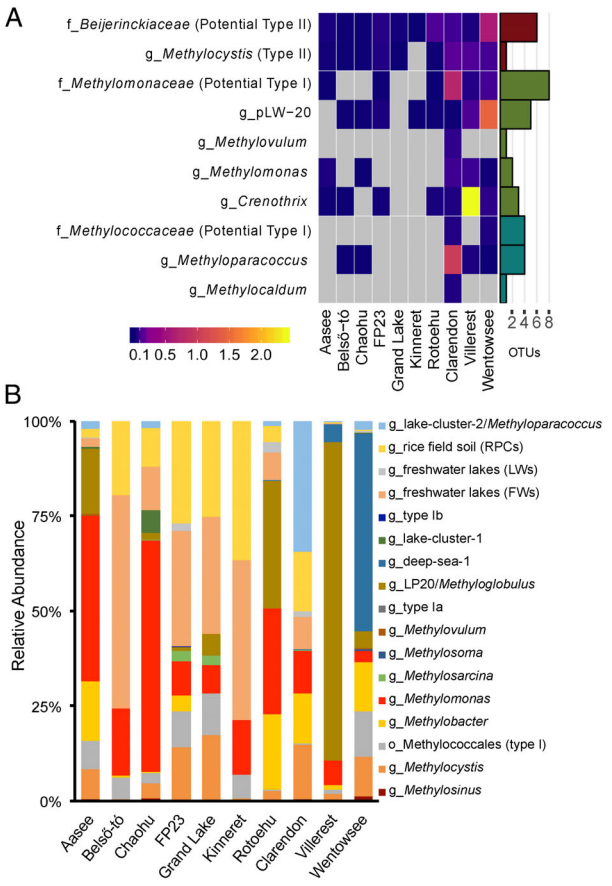

Fig. 3. Distribution of methanotrophic lineages across 10 lakes. The lakes are arranged with the three lakes with the highest number of methanotrophs on the right. The following taxonomic levels are used: o – order, f – family, g – genus.
A. The relative abundance of methanotrophic lineages in the non-cyanobacterial community as revealed by bacterial 16S rRNA gene sequencing. The scale bar shows the fraction of each lineage. A grey colour indicates that the relative abundance was below 0.005%. The right bars are coloured by family *Beijerinckiaceae* (red), *Methylomonaceae* (green), *Methylococcaceae* (blue) and represent the number of OTUs within each group.
B. The relative abundance of methanotrophic lineages in methanotrophic communities as revealed by *pmoA* sequence analysis. [Color figure can be viewed at wileyonlinelibrary.com]

Despite our ability to detect MOB through 16S rRNA gene analysis, most of the sequencing results are related to the predominant Cyanobacteria, and the 16S rRNA gene of rare methanotrophs is not fully recovered. Therefore, the derived diversity estimations are less statistically valid. Thus, we also sequenced the *pmoA* functional marker genes from all samples to more effectively characterize the diversity of the MOB community, as *pmoA* primers being highly specific, can more sensitively detect rare methanotrophs. The amplicon yield of *pmoA* genes ranged from 976 sequences in Belső-tó to 132 557 in Lac de Villerest, with a total of 303 339 sequences clustered into 125 OTUs. The uncultivated lineages are identified in the context of a phylogenetic tree and named according to the habitat where they are predominantly found or first reported (Dumont *et al*., 2014). By comparing the *pmoA* sequences from all 10 lakes to reference sequences of each lineage provided by a recent study (Dumont *et al*., 2014), we obtained seven uncultivated lineages at the genus level. Notably, three different lineages appeared to be the most abundant methanotrophs in each of the three high methanotroph lakes ('lake-cluster-2' constituted 34.4% of pmoA sequences in Lake Clarendon; 'LP20' made up 83.9% in Lac de Villerest; and 'deep-sea-1' made up 51.9% in Wentowsee) (Fig. 3B). These three lineages were present at low levels or not at all in the other lakes. 'LP20' and 'lake-cluster-2' are, respectively, related to two recently defined genera *Methyloglobus* and *Methyloparacoccus* (as the dominant *pmoA* OTUs from each of the uncultivated lineages was 96% similar to the related type strains) and supported by updated classifications in a recent study (Knief, 2015). Among the sequences related to cultivated bacteria, common freshwater genera including *Methylocystis*, *Methylomonas* and *Methylobacter* were commonly observed (Fig. 3B). Unfortunately, we were unable to detect the *pmoA* gene of *Crenothrix* sp., uncovered by 16S rRNA gene analysis in Lac de Villerest, due to its atypical gamma-proteobacterial *pmoA*, which mismatched with the primers used here (Knief, 2015).

As guided by the strengths of each marker gene, we estimated the diversity indices and the abundance of MOB communities using *pmoA* and 16S rRNA gene sequences, respectively. The MOB members together accounted for over 2% of the non-cyanobacterial communities in each of the three lakes (Lake Clarendon, Lac de Villerest, and Wentowsee), levels of which were approximately 10- to 100-fold higher than in the other seven lakes. Based on these order of magnitude differences, 10 lakes were correspondingly grouped into 'high' and 'low' MOB categories (Table 1). Our inability to detect *Crenothrix* sp. in Lac de Villerest by targeting *pmoA*, likely contributed to the unexpectedly low Shannon diversity in this 'high' group lake (Table 1).

Quantitative and qualitative relationships of methane metabolizing microbes

To quantify the relationship between methane producers and consumers in *Microcystis* aggregates, qPCR was used in all five lakes where both groups were present. The abundance of each of the two groups, as well as *Microcystis*, was first estimated as cell numbers per μg of DNA (Supporting Information Fig. S7). To facilitate comparisons of methanogens and methanotrophs in a more meaningful way, their original abundances were further normalized by dividing their cell numbers by those of

Table 1. The diversity indices of methanotrophic communities and their relative abundance within the non-cyanobacterial community at each lake.

Groups[a]	Lakes	Diversity indices[b]			Relative abundance[c]
		Observed_OTUs	Shannon	Phylogenetic[d]	
Low	Aasee	45.7 ± 4.2	3.6 ± 0.3	6.8 ± 0.4	0.100% ± 0.037%
	Belső-tó	4.0 ± 1.4	1.3 ± 0.2	1.6 ± 0.4	0.044% ± 0.017%
	Chaohu	10.7 ± 4.6	1.9 ± 0.1	2.8 ± 0.6	0.050% ± 0.004%
	FP23	13.3 ± 2.4	2.8 ± 0.5	3.3 ± 0.3	0.162% ± 0.048%
	Grand Lake	10.0 ± 2.2	2.6 ± 0.2	2.9 ± 0.3	0.038% ± 0.004%
	Lake Kinneret	3.7 ± 1.2	1.4 ± 0.4	1.6 ± 0.4	0.019% ± 0.002%
	Lake Rotoehu	28 ± 7.3	3.6 ± 0.3	4.9 ± 0.9	0.194% ± 0.019%
High	Lake Clarendon	31.7 ± 2.9	3.2 ± 0.2	5.4 ± 0.3	2.296% ± 0.416%
	Lac de Villerest	61.3 ± 15	1.9 ± 0.6	8.6 ± 1.7	2.976% ± 0.287%
	Wentowsee	69.7 ± 23.3	3.7 ± 0.3	9.5 ± 2.6	2.355% ± 0.262%

[a]Lakes were grouped into two categories: low – seven lakes with a low relative abundance of methanotrophs; high – three lakes with a higher relative abundance of methanotrophs.
[b]Estimation was based on *pmoA* sequence analysis.
[c]Estimation was based on 16S rRNA gene sequence analysis.
[d]Phylogenetic diversity determined using Faith's PD whole tree.

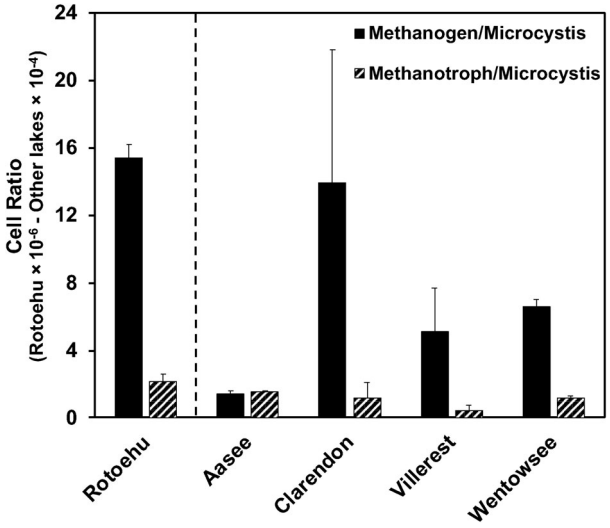

Fig. 4. The normalized abundance of methanogen and methanotroph cell numbers relative to *Microcystis* cell numbers from five lakes where methanogens were detected. *Microcystis* was enumerated by qPCR of its 16S rRNA gene, methanogens using *mcrA* and methanotrophs using *pmoA* on bloom aggregate samples.

Microcystis (Fig. 4). In Lake Rotoehu, with the lowest levels, there were 15.4 ± 0.8 methanogen cells and 2.1 ± 0.4 methanotroph cells per million *Microcystis* cells (Fig. 4), while in the other four lakes, methanogen numbers ranged from 140 ± 20 (Aasee) to 1390 ± 790 (Lake Clarendon), and methanotroph numbers from 50 ± 20 (Lac de Villerest) to 150 ± 10 (Aasee) cells per million *Microcystis* cells (Fig. 4). The abundance of methanotrophs is likely underestimated in Lac de Villerest due to our failure to detect the *Crenothrix* sp. *pmoA*. Linear regression was further conducted to estimate the abundance relatedness between methanotrophs and methanogens or *Microcystis* and demonstrated that cell numbers of methanotrophs were positively correlated with cell numbers of methanogens ($R^2 = 0.4$, $P < 0.05$) (Fig. 5A). In contrast, cell numbers of *Microcystis* showed no correlation ($R^2 = 0.1$, $P = 0.34$) (Fig. 5A). Likewise, there was a strong positive correlation between the number of *pmoA* OTUs and the number of *mcrA* OTUs across all 10 lakes ($R^2 = 0.56$, $P < 0.0001$), but only a weak correlation with the number of cyanobacterial 16S rRNA OTUs ($R^2 = 0.26$, $P < 0.05$) (Fig. 5B).

Heterotrophic bacteria associated with methanotrophs within the aggregate

Interactions between methanotrophs and heterotrophs are widespread in natural systems (Ho *et al*., 2016). It is therefore reasonable to speculate that methanotrophs could have indirect effects on aggregate community function through interacting with the associated heterotrophs. The potential interacting heterotrophs are likely to be co-enriched with methanotrophs in the three high MOB lakes and abundances should be strongly correlated with methanotroph abundances. Following this assumption, we first attempted to verify whether heterotrophic communities in high MOB lakes were dissimilar from those in low MOB lakes. Significant differences (but with less strength) in the non-cyanobacterial heterotrophic communities was identified between the high and low MOB lakes using three nonparametric multivariate analysis methods (Supporting Information Table S2). As reflected on PCoA analysis, high MOB lakes clustered together and were marginally separated from low MOB lakes along the third axis (Supporting Information Fig. S8). In agreement with the observed weak dissimilarity, 30 major family-level lineages were found to differ significantly ($P < 0.05$) in abundances between the two groups of lakes (Fig. 6A).

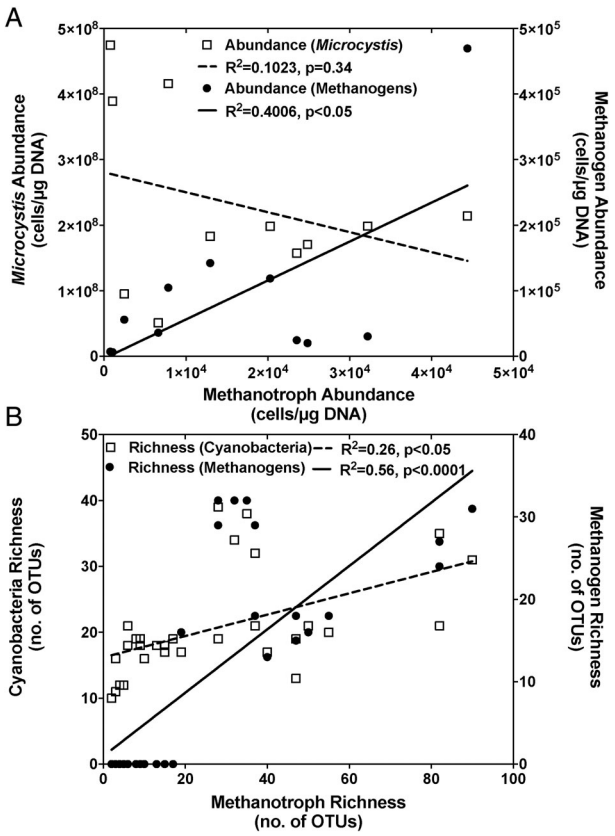

Fig. 5. The abundance (quantitative) and richness (qualitative) of methanogens were both positively and linearly correlated with that of methanotrophs within bloom aggregate samples.
A. Linear regression of abundance between methanotroph cells and either methanogens or *Microcystis* cells. Calculations were based on the five lakes where all three types of organisms co-localized.
B. Linear regression of richness between methanotrophs and either methanogens or Cyanobacteria. Calculations included three individual samples from each of 10 lakes. The methanotrophs and methanogens were enumerated by targeting *pmoA* and *mcrA*, respectively, while Cyanobacteria and *Microcystis* were enumerated based on the 16S rRNA gene. Richness data were based on sequence analysis and abundance using qPCR.

Among them, 15 heterotrophic families were significantly more abundant in the high MOB lakes and also positively correlated with the abundance of methanotrophic lineages (Fig. 6B).

Discussion

Presence of methanogenic archaea in Microcystis *aggregates*

Previous studies have shown that *Methanoregula*, *Methanosaeta* and uncultured *Methanomicrobiales* were the only methanogenic lineages exclusively associated with the particle fractions of various *Microcystis* strains in lab cultures (Batista *et al*., 2019) or planktonic photoautotrophs in an oligo-mesotrophic lake (Grossart *et al*., 2011). Six OTUs belonging to uncultured Methanomicrobiales were similarly observed in the *mcrA* gene results (Fig. 2), indicating that this order likely contains additional novel lineages adapted to cyanobacterial aggregates. The results presented here further revealed the diversity of methanogenic communities by identifying the less common lineages: Methanobacteriales, Methanocellales, Methanomassiliicoccales and Methanofastidiosales. Aggregates may provide protective habitats and favourable (temporally) anoxic niches, providing support for the notion that methanogenic archaea rarely persist as free-living organisms in surface waters (Grossart *et al*., 2011, Batista *et al*., 2019). Production of acetate, H_2 and CO_2 by *Microcystis* strains under dark conditions through intracellular fermentation of stored photosynthates may provide energy for acetoclastic and hydrogenotrophic methanogens in aggregates (Yasuo and Kawamura, 1984; Moezelaar and Stal, 1994; Dziallas and Grossart, 2012). Alternatively, N_2-fixation could be an important source of H_2 in planktonic aggregates (Paerl and Pinckney, 1996), and N_2-fixing bacteria have been shown to be important components of *Microcystis* aggregates (see below). Methanogens may also be important in N_2 fixation within the aggregates as methanogenic Euryarchaeota are the only known archaea that contain *nif* genes (Leigh, 2000). In the Florida Everglades, methanogenic Euryarchaeota are major contributors to N_2 fixation in soils (Bae *et al*., 2018). The absence of detectable methanogens or other Archaea in five of the lakes suggests that other factors may influence the ability of methanogens to survive within the cyanobacterial aggregate, as well as affect their community composition (Supporting Information Fig. S5c). These factors might include the presence of alternative respiratory electron acceptors (sulfate, nitrate) in lakes, which could allow the methanogens to be out-competed by other anaerobic respiring bacteria (Thauer *et al*., 2008). This competition is becoming important as many lakes now have increasing sulfate (Tao *et al*., 2013) and nitrate concentrations. An increase in levels of sulfate reducing bacteria within Desulfovibrionales (i.e., Chaohu) (Supporting Information Fig. S9) and high concentrations of nitrate and nitrite (i.e., Belső-tó and FP23) (Supporting Information - Table S3) observed in lakes lacking methanogens, support this hypothesis. Alternatively, another factor that could control methanogenic viability within aggregates is the presence/absence of enzymes capable of oxygen radical degradation in other microorganisms, which might protect methanogens against oxidative stress under high light conditions (Kim *et al*., 2019).

Co-occurrence of methanogen-associated archaea

Along with methanogenic Euryarchaeota, the other abundant archaeal classes Woesearchaeia and Bathyarchaeia

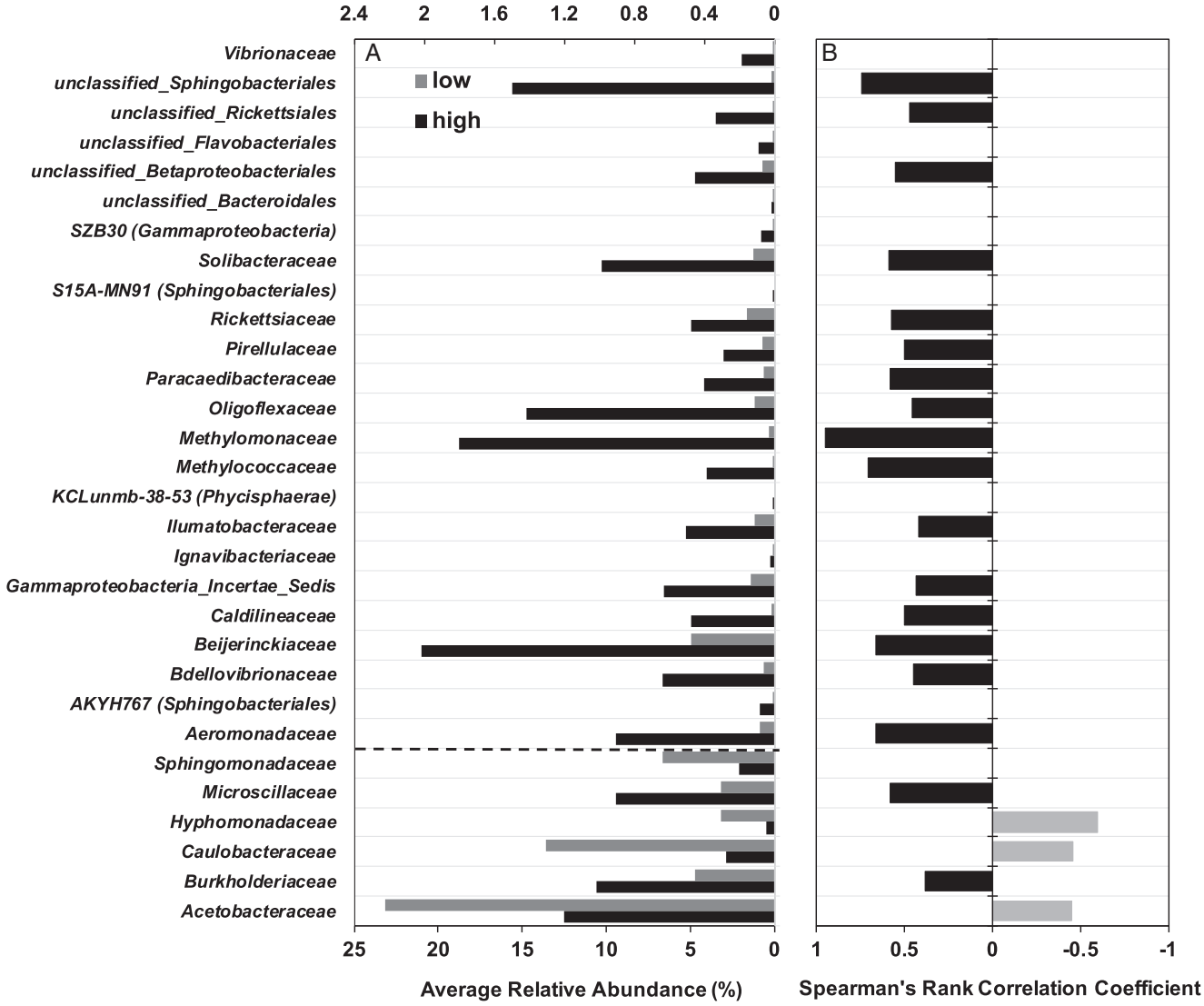

Fig. 6. Bacterial families potentially interacting with methanotrophs. Note that X-axes increase from right to left to allow visualization of groups.
A. Bacterial families with significant differences in abundance (in the non-cyanobacterial community) between the high- and low-group lakes. The bacterial families with $P < 0.05$ (tested using nonparametric *t*-test) and average relative abundance >0.1% in non-cyanobacterial community are listed. The highly abundant families are below the dashed line and use the bottom X-axis with the others using the upper X-axis.
B. Spearman's rank correlation of the abundance between these families and methanotrophs. Black bars show a positive correlation while the grey bars show a negative correlation. The coefficient rho value from 0 to ± 1 in the bottom axis denote the degree of the correlation from weak to strong. Only the families with correlation significances of $P < 0.05$ are shown.

likely interact with methanogens. Woesearchaeia-methanogen consortia are globally distributed in inland anoxic environments with a syntrophic relationship proposed based on their genomic features (Liu *et al*., 2018). Members of Bathyarchaeia are also widespread in anoxic environments most likely due to a range of metabolic capabilities, including a potential for methane metabolism (Evans *et al*., 2015) and acetogenesis (He *et al*., 2016), likely feeding reducing equivalents to methanogens during anoxic periods. Together, Euryarchaeota, Woesearchaeia and Bathyarchaeia made up 96.4%–99.3% of the archaeal communities across the five lakes, highlighting the potential importance of Archaea in methane-related carbon flow in cyanobacterial aggregates.

Global distribution of MOB communities in cyanobacterial bloom aggregates

In order to fully understand the role of MOB in cyanobacterial bloom aggregates, it is critical to understand their distribution on a global scale. This worldwide survey of bacterial 16S rRNA genes showed that proteobacterial MOB were ubiquitously present in *Microcystis* aggregates but most abundant in 3 of the 10 lakes

(i.e., the high MOB lakes). One of a variety of environmental parameters or the availability of methanotroph substrates (i.e., CH_4, O_2 and C_1-compounds) could directly or indirectly affect the distribution of methanotrophic genera in the lakes. Although C_1 methylated compounds are potential substrates for MOB, they are commonly found at low concentrations in the oxic epilimnion of lakes (Watson and Juttner, 2017). Diurnal O_2 fluctuation (high during the day and low at night) and its relative importance at specific times of the bloom season (Chen *et al*., 2016), likely influence the taxonomic composition and allow for alpha-MOB (see below) to dominate as these are thought to be better adapted to fluctuating O_2 concentrations in surface waters (Reis *et al*., 2020). Although limited lake methane data is available, Chaohu recorded a relatively high CH_4 concentration (0.36 μM CH_4), compared to the lower CH_4 levels (<0.1 μM) recorded in two of the five lakes where methanogens occurred (Supporting Information Table S3). This provides some support for the argument that in the five lakes where methanogenic archaea are present (see below), methane is cycled internally with little spillover to the surface water. On the other hand, MOB in the other five lakes may be fed by CH_4 diffusing from outside of aggregates. However, more information is needed to conclusively determine this.

Environmental effects were also considered. The higher dissolved organic carbon (DOC) concentrations observed in Lake Clarendon and Wentowsee (Supporting Information Table S3) may have increased the activity and abundance of MOB by relieving light inhibition in surface waters (Thottathil *et al*., 2018) or by providing carbon for anaerobic bacteria living within the aggregates to produce methane. Previous results from a high MOB lake (Lac de Villerest) along with two low MOB lakes (Grand Lake and Lake Kinneret), showed MOB present in their mid-water column where diffusive CH_4/O_2 countergradients meet during the stratification period (Junier *et al*., 2010; Morrison *et al*., 2017; Pradeep Ram *et al*., 2019). Considering that Lac de Villerest was sampled during the early period of turnover (October), while the other two lakes were sampled at the beginning of the stratification period (Supporting Information Table S3), we speculate that abundant MOB may have been circulated to the surface during the fall turnover in Lac de Villerest and subsequently recruited into bloom aggregates.

Although there have been few studies specifically focusing on MOB associated with cyanobacterial blooms, MOB-related lineages have been previously reported in *Microcystis*-bloom containing lakes (Yang *et al*., 2017; Akins *et al*., 2018; Guedes *et al*., 2018; Yang *et al*., 2019). For example, the eutrophic Dianchi had a higher abundance of MOB in sediments in comparison to a nearby mesotrophic lake (Yang *et al*., 2019). In another study, members of the MOB lineage Methylococcales were found to be present in the water column and temporally associated with *Microcystis* in Funil Reservoir (Brazil) (Guedes *et al*., 2018).

Analysis of both 16S rRNA and *pmoA* genes showed that type I gamma-MOB represent abundant methane oxidizers in *Microcystis* aggregates. As type I MOB are often more abundant than type II MOB in lake waters (Biderre-Petit *et al*., 2011; Blees *et al*., 2014; Kojima *et al*., 2014; Samad and Bertilsson, 2017), it is possible that MOB within the aggregates were recruited from the water column. For example, *Methyloparacoccus* (referred to as 'lake-cluster-2' in Fig. 3B) was the most abundant MOB in Lake Clarendon and was recently isolated from pond water in South Africa and Japan (Hoefman *et al*., 2014b). Related clones have also been observed in several lakes where *Microcystis*-blooms are present, including Dianchi (Yang *et al*., 2019), Taihu (Wu *et al*., 2007) and Kastoria Lake (Kormas *et al*., 2010). In Wentowsee, pLW-20 and 'deep-sea-1' were the most abundant MOB lineages as determined by 16S and *pmoA*, respectively. The predominant OTU in each of these lineages was most closely related to the 16S rRNA sequence (94% nucleotide sequence identity) and *pmoA* sequence (93% amino acid sequence identity) of *Methylobacter marinus A45* (Lidstrom, 1988; Bowman *et al*., 1993) which requires NaCl for growth and may be capable of N_2 fixation (Flynn *et al*., 2016). *Methylocystis* was the only MOB genus, verified by both primer sets, to be present in all lakes. Both *pmoA* (98.7% amino acid sequence identity) and 16S rRNA gene (99.8% nucleotide sequence identity) derived OTUs were most closely related to those of *Methylocystis parvus*, with the ability to form lipid cysts as a resting stage (Whittenbury *et al*., 1970; Bowman *et al*., 1993) and to intracellularly ferment hydroxybutyrate under anoxic conditions (Vecherskaya *et al*., 2009). These and other potential adaptive traits may explain their ubiquitous distribution pattern. The detection of the 16S rRNA gene of *Crenothrix* sp. in Lac de Villerest was consistent with previous findings that this filamentous MOB was more abundant in *Microcystis*-associated aggregates than free living in the water column (Yang *et al*., 2017). Our *pmoA* results indicate that the *Methyloglobulus*-like lineage 'LP20' represents another important MOB group in Lac de Villerest. *Methyloglobulus* has been described to grow optimally at reduced oxygen concentrations (Deutzmann *et al*., 2014a). This suggests that these MOB communities are well adapted to life in *Microcystis* aggregates, where high nutrients (Yang *et al*., 2019) and reduced oxygen concentrations (Blees *et al*., 2014; Deutzmann *et al*., 2014a; Oswald *et al*., 2017) as well as particle-associations (Yang *et al*., 2017) are common.

It is important to note that, under anoxic conditions, methane-dependent nitrate and nitrite reduction can be, respectively, catalysed by ANME-2d associated archaeal methanotrophs (Haroon *et al*., 2013) and the bacterial genus *Methylomirabilis* in phylum NC10 (Ettwig *et al*., 2008). Not surprisingly, neither *Methylomirabilis*-like 16S rRNA gene sequences (Fig. 3A) nor ANME-2d associated *mcrA* (Fig. 2) or related 16S rRNA gene sequences (Fig. 1) were retrieved from any aggregate samples, whereas positive control sediments and filters effectively amplified these sequences (data not shown). These observations concur with the lack of evidence for the occurrence of anaerobic methanotrophs in surface waters, presumably due to the slow growth rate (doubling time: $T_d > 1$ month; Kampman *et al*., 2012; Zhu *et al*., 2012; Vaksmaa *et al*., 2017) restricting them to habitats with lower turnover, such as the lake and groundwater sediments (Deutzmann *et al*., 2014b). Acidophilic methanotrophs from the phylum Verrucomicrobia (Dunfield *et al*., 2007) were also not detected in aggregates (Fig. 3A), perhaps due to the unfavourable neutral to alkaline pH that occurs throughout the bloom season.

MOB capable of directly influencing the N-cycle in bloom aggregates

Microcystis, as a non-diazotrophic cyanobacterium, is solely reliant on fixed N sources derived from lake water or from N-cycling reactions conducted by its associated bacteria (Cook *et al*., 2020). The role of N_2-fixing bacteria becomes critical during the midsummer period of maximal bloom growth, during which dissolved inorganic N decreases dramatically (Xu *et al*., 2010; Jankowiak and Gobler, 2020). As such, Rhizobiales, Azospirillales (Supporting Information Fig. S9) (Yang *et al*., 2017; Kim *et al*., 2019; Jankowiak and Gobler, 2020) and many MOB lineages having N_2-fixation potential (Auman *et al*., 2001), could increase N availability to *Microcystis*. Possible methanotrophic taxa could include members from the abundant genera *Crenothrix* (Oswald *et al*., 2017), *Methyloglobus* (Deutzmann *et al*., 2014a), *Methylicorpusculum* (Saidi-Mehrabad *et al*., 2020), and also ubiquitous genera such as *Methylobacter* (Kalyuzhnaya *et al*., 2015; Flynn *et al*., 2016), *Methylomonas* (Hoefman *et al*., 2014a; Kalyuzhnaya *et al*., 2015), and *Methylocystis* (Whittenbury *et al*., 1970; Bowman *et al*., 1993). O_2-depleted microzones are usually required for facilitating N_2-fixation in cyanobacterial aggregates (Paerl and Bebout, 1988; Paerl and Pinckney, 1996). Daylight promotes photosynthesis resulting in high O_2 levels, whereas dark respiration of organic compounds contributes to O_2 depletion (Dziallas and Grossart, 2012; Klawonn *et al*., 2015). As such, N_2-fixation in cyanobacterial aggregates has been shown to be far more active during darkness (Klawonn *et al*., 2015).

Likewise, the presence of anoxic or suboxic microzones could help us understand the occurrence of previously reported aggregate-associated denitrifying bacteria (Yang *et al*., 2017; Jankowiak and Gobler, 2020). Many of the MOB genera found here are also known to contain genes encoding enzymes involved in denitrification (Hoefman *et al*., 2014b; Smith *et al*., 2018; Saidi-Mehrabad *et al*., 2020) and methane oxidation coupled to denitrification occurs in type I MOB under hypoxia (Kits *et al*., 2015a, b). *Crenothrix* and *Methylobacter* probably take advantage of denitrification to grow within the oxygen-deficient water column (Oswald *et al*., 2017; van Grinsven *et al*., 2020). However, denitrification reactions in cyanobacterial aggregates require N oxides to diffuse from oxic zones where nitrification occurs (Klawonn *et al*., 2015). Nitrifying bacteria (*Nitrosomonadaceae*) were present in all *Microcystis* aggregates in all sampled lakes and were observed in other studies (Louati *et al*., 2015; Jankowiak and Gobler, 2020) suggesting a likely source of N-oxides.

Potential coupling of methane production and consumption between methanogenic archaea and MOB

Given the co-occurrence of MOB and methanogenic archaea within *Microcystis* aggregates, the question remains as to whether their metabolisms are coupled. Previous work has demonstrated a positive relationship between biodiversity and ecosystem function on highly specialized metabolic processes carried out by individual groups of microbes (Wohl *et al*., 2004; Peter *et al*., 2011). For example, diversity indices and absolute abundance of MOB have been demonstrated to be positively correlated with methane consumption rates in a wetland (Bodelier *et al*., 2013), upland soil (Levine *et al*., 2011) and in surface lake waters (Reis *et al*., 2020). In laboratory studies with cyanobacteria-associated soil aggregates, the abundance of methanogenic archaea was positively correlated with the methane production rate (Angel *et al*., 2012). Our results also reveal a positive correlation between methanogens and MOB total numbers as well as between methanogen and MOB richness, suggesting that the activities of both groups are indeed coupled.

In order for methanogenesis and methanotrophy to be coupled, both would likely occur in overlapping time scales. Most methanogenic archaea are known to be sensitive to O_2. Several groups, however, can tolerate O_2 exposure (Huser *et al*., 1982; Jarrell, 1985) but are most likely only active under highly reduced conditions. A number of studies have suggested that *Crenothrix* (Oswald *et al*., 2017), *Methylobacter* (Biderre-Petit *et al*., 2011; Blees *et al*., 2014; van Grinsven *et al*., 2020) and unclassified lineages of gamma-MOB (Oswald *et al*., 2016; Cabrol *et al*., 2020) are abundant and active in O_2-depleted lake

water columns. The most abundant *pmoA* OTU in Wentowsee was most closely related to clones (HF674404: 98.7% and AB930855: 96.8% similarity) previously obtained from a deep anoxic lake water layer (Blees *et al*., 2014; Kojima *et al*., 2014). Presumably, these MOB are also growing in low O_2 environments using a combination of aerobic and anaerobic (nitrate/nitrite respiration or fermentation) metabolisms (Vecherskaya *et al*., 2009; Kalyuzhnaya *et al*., 2013; Oswald *et al*., 2017; van Grinsven *et al*., 2020). For temporal alignment of these two groups, both methanogens and MOB would likely cooperate within microzones exhibiting fluctuating oxygen conditions with low concentrations during darkness and high concentrations when light is present. Previous work also suggests that daytime light results in a lower methane oxidation rate, due to photoinhibition of MOB (Murase and Sugimoto, 2005; Tang *et al*., 2014; Tang *et al*., 2016; Thottathil *et al*., 2018). It is therefore likely that darkness is the niche for temporal coupling of MOB and methanogenic archaea activities to co-occur within *Microcystis* aggregates. While our analysis is based entirely on relative abundances, direct measures of coupled activities will be required to address in detail the potential for methanogenesis and methanotrophy to occur in the cyanobacterial aggregates, even though such work will be a real challenge to perform in such a dynamic microscale system.

Coupling of methanogenic archaea activities could potentially provide MOB access to much higher methane concentrations than might be available solely by diffusion from anoxic benthic waters and sediments (Bastviken *et al*., 2011). Several possible alternative mechanisms for microbial methane production could exist within cyanobacterial aggregates (Tang *et al*., 2014; Tang *et al*., 2016; Bizic *et al*., 2020). However, none of the alternatives could produce methane at levels that compare to those of active archaeal methanogens (Bizic *et al*., 2020), indicating that within anoxic/suboxic cyanobacterial aggregates where methanogenic archaea are present, they represent an important source of methane, in particular when oxygen levels are low. In their absence and at increasing oxygen concentrations, alternative sources of methane such as from cyanobacterial oxic methane production will be more important.

MOB: a potential hidden driver of carbon flow within cyanobacterial aggregates

The co-occurrence and co-variation of methanotrophic and non-methanotrophic bacteria in cyanobacterial aggregates can be used to predict the existence of ecological interactions among various microorganisms. For example, abundance-based correlations have predicted that redox partnerships exist between MOB and their associated microbiome in anoxic waters (Cabrol *et al*., 2020). Here, the abundance (based on relative read levels) of the three methanotroph-containing families (*Methylomonaceae*, *Methylococcaceae* and *Beijerinckiaceae*) (Fig. 6) were significantly higher in the high MOB lakes, supporting our results on the separation of 10 lakes into two groups. Several families identified here to be enriched with methanotrophs (e.g., *Microscillaceae*, *Aeromonadaceae*, *Caldilineaceae* and *Solibacteraceae*) are chemo-organotrophs and usually present in organic compound enriched habitats, where their functional potentials are not well understood. Previous stable isotope probing studies have suggested that members of the *Burkholderiaceae* and *Bdellovibrionaceae* may feed either on metabolites released during methane oxidation or directly on cells of MOB in natural consortia (Morris *et al*., 2002; Ho *et al*., 2016), explaining their associations observed in this study. Along with Bacteria and Archaea, methane-derived carbon can also be assimilated into the biomass of grazing Eukarya (Murase and Frenzel, 2007), which could be further utilized by obligate intracellular parasitic bacteria from *Paracaedibacteraceae* (Hess *et al*., 2016) and *Rickettsiaceae* (Gortz and Brigge, 1998), two other groups enriched in *Microcystis* aggregates of high MOB lakes. The heterotrophic family *Ilumatobacteraceae* also enriched here was found to be enriched with *Methyloglobulus* in bathypelagic water layers of Lake Baikal (Cabello-Yeves *et al*., 2020), suggesting a functional or evolutionary relationship. Such cross-feeding interactions allow us to hypothesize that a heterotrophic food web dependent on the additional carbon contributed by MOB could be present.

The co-occurrence of methanogens, nitrifiers and specific heterotrophs with MOB at the scale of hundreds of micrometres within cyanobacterial aggregates suggests to us that their functions interact to directly link the carbon and nitrogen cycles as illustrated in Fig. 7. These interactions could allow a stable and co-evolving population of organisms to thrive within the aggregates (Garcia *et al*., 2015).

In conclusion, our results provide compelling molecular evidence for the co-occurrence of methanotrophs and methanogenic archaea in *Microcystis* aggregates from a global perspective. The absolute abundances and richness of MOB communities and methanogenic communities were positively correlated across global lake samples, suggesting that methane cycling is important within these cyanobacterial aggregates. Yet, their *in situ* activities and interactions require further exploration.

Experimental procedures

Bloom samples and collection

Cyanobacterial bloom samples dominated by *Microcystis* were collected from 12 lakes (Cook *et al*., 2020). Here, only 10 of those lakes were used for studying methane metabolizing organisms within *Microcystis* aggregates

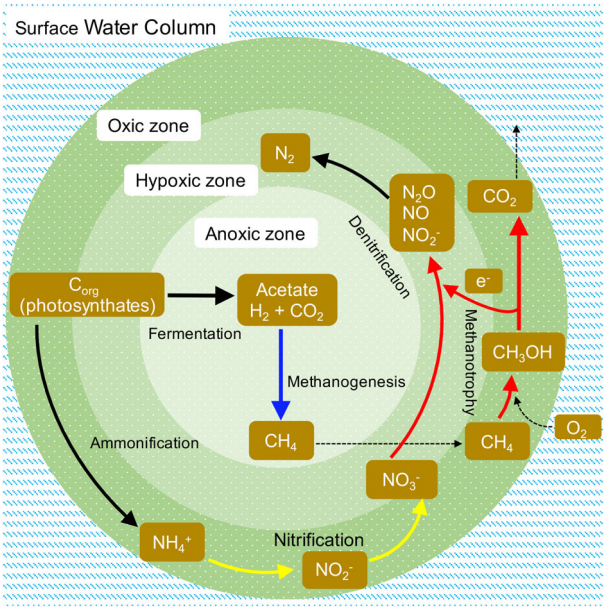

Fig. 7. Schematic overview demonstrating how methanotrophs potentially act as an interconnecting bridge between the C and N cycle in *Microcystis* aggregates. This schematic depicts an aggregate functioning in the absence of light. Dashed arrows indicate diffusion processes. Solid arrows indicate metabolic pathways, which could be catalysed by methanogens (blue)/methanotrophs (red)/nitrifiers (yellow)/others (black). Pathways are discussed in the text. [Color figure can be viewed at wileyonlinelibrary.com]

because the other two lakes were missing replicates. The 10 lakes spanned a 90° latitudinal and 270° longitudinal gradient (Supporting Information Fig. S1) and their general limnological information was included as well (Supporting Information Table S3). It is important to note that this information is a summary, based on recently recorded data and in most cases, was not directly collected during sampling trips. The size of lakes varies greatly, from 0.001 (FP23) to 770 km^2 (Chaohu). The five lakes where methanogens were present appear to be restricted to high-latitude regions. Methane, DOC and stratification data were used to help explain the differential distribution of methanotrophs (see above).

For collection of large *Microcystis* aggregates, surface water samples were collected and left undisturbed for 10 min. The concentrated floating cyanobacterial biomass on the surface was then filtered through triplicate sterile Nitex screens (100-μm pore size). The Nitex screens were then submerged in DNA preservative (Zymo Research, Irvine, CA, USA), shipped at ambient temperature and stored at −20°C prior to DNA extraction (for details, see Cook *et al*., 2020).

DNA extraction, amplification and Illumina sequencing

DNA was extracted from Nitex screens using Quick-DNA Faecal/Soil Microbe Miniprep Kits (Zymo Research). The amplicons of bacterial 16S rRNA genes sequenced in our previous study (Cook *et al*., 2020) were used for exploring the methanotrophic community, since *in silico* evaluation results indicated that the primers (Supporting Information Table S4a) used, had broad coverage of bacterial methanotrophic lineages (Table S4b). In this study, the archaeal 16S rRNA, *mcrA* (targeting methanogenic Archaea) and *pmoA* (targeting methanotrophic Bacteria) genes were amplified from the same DNA template using the corresponding primers and thermocycler conditions as summarized in the Supporting Information Table S4a. For each of the purified amplicons, 5 μl was added to a second PCR mixture containing the appropriate index primers using the above PCR conditions for another eight cycles, to allow each sample to receive a unique 'barcodeF + barcodeR' combination sequence to allow demultiplexing post-pooling. The mixed sample was sequenced on an Illumina MiSeq platform at the Oklahoma Medical Research Foundation (300 bp paired-end).

Quantitative real-time PCR

Quantification of methanogens, methanotrophs and *Microcystis* abundance was performed via qPCR, targeting *mcrA*, *pmoA* and 16S rRNA genes *of Microcystis*. All qPCR assays were run on a CFX96 Touch RT-PCR System (Bio-Rad Laboratories, Hercules, CA, USA) with the procedures provided in the Supporting Information Table S4a. Standard curves were created by serially diluting known amounts of PCR products of 16S rRNA, *mcrA* and *pmoA* genes from *Microcystis aeruginosa*, *Methanospirillum hungatei* and *Methylosinus trichosporium*, respectively, cloned into the pCR4-TOPO vector (Invitrogen, Carlsbad, CA, USA) and amplified with vector-specific primers. Efficiency (E) and R^2 were calculated from the standard curve using the Bio-Rad CFX Maestro software and summarized for each target gene: $E = 80.6\%$, $R^2 = 0.999$ (16S rRNA); $E = 90.7\%$, $R^2 = 0.993$ (*mcrA*); $E = 97.5\%$, $R^2 = 0.941$ (*pmoA*).

Sequence processing and analysis

Paired-end reads were merged with USEARCH (Edgar, 2010) with the length restricted to 400–550 bp and a maximum error set to 1. The joined fragments with undetermined nucleotides were removed, and fragments were truncated where there were three consecutive low-quality nucleotides (quality score < 20), followed by discarding sequences that had less than 75% of their original length.

For archaeal 16S rRNA gene sequences, the remaining high-quality fragments were de-replicated and clustered into OTUs at the 97% similarity level with UPARSE (Edgar, 2013). Taxonomic annotations were

assigned to each OTU sequence with the RDP Classifier (Wang *et al*., 2007) against the SILVA-SSUv132 Database (Quast *et al*., 2013), and the OTUs annotated as Bacteria were removed. The samples with low sequencing depth and OTUs with an abundance below 0.005% were discarded. The potential methanogenic OTUs were identified based on the recent review on classification of methanogens (Evans *et al*., 2019).

For *mcrA* and *pmoA* sequences, the remaining high-quality sequences that did not match (using BLAST) a reference collection of *mcrA* (Euryarchaeota and Verstraetearchaeota) or bacterial *pmoA* sequences at a cutoff e value of 1e-10 were discarded. Functional gene OTUs were picked with UCLUST (Edgar, 2010) in QIIME (Caporaso *et al*., 2010) at a minimum sequence identity of 84% (*mcrA*) and 87% (*pmoA*) (Degelmann *et al*., 2010; Yang *et al*., 2014). The OTU nucleotide sequences were aligned with MAFFT (Katoh and Standley, 2013) and phylogenetic distances were calculated with QIIME. For the *pmoA* gene, taxonomic assignments of OTUs were made with UCLUST against Dumont's *pmoA* database (Dumont *et al*., 2014). To obtain detailed taxonomic assignments and phylogenetic relationships from *mcrA* OTUs, representative amino-acid sequences (translated with FrameBot; Wang *et al*., 2013) from each OTU were extracted for performing a web-based BLAST search to retrieve closer relatives, which were combined into the reference collection mentioned above. After aligning all the sequences with MAFFT, the maximum likelihood phylogenetic tree of *mcrA* amino-acid sequences was constructed with RAxML 8.2.12 (Stamatakis, 2014) and organized in ARB v6.0.4 (Ludwig *et al*., 2004).

To determine the composition of methanotrophic communities among lakes using bacterial 16S rRNA genes, we used the non-cyanobacterial OTU table generated previously (Cook *et al*., 2020). The gamma-MOB were affiliated with three families (*Methylococcaceae*, *Methylomonaceae* and *Methylothermaceae*), while the alpha-MOB have recently been merged into *Beijerinckiaceae* (Parks *et al*., 2018). The OTU sequences of these families were extracted from the table (Cook *et al*., 2020).

Statistical analyses

Phylogenetic structure dissimilarities were compared for methanogenic communities in the five lakes using the weighted UniFrac distance and displayed in principal coordinates analysis (PCoA) plots. Procrustes analysis (Peres-Neto and Jackson, 2001) was conducted to determine the similarity of the PCoA between pairs of samples from 16S rRNA and functional gene datasets. The analysis provided a m^2 value to demonstrate similarity between the two datasets and associated *p*-value based on 999 Monte Carlo simulations. Non-parametric tests and PCoA plot were used to assess and display the phylogenetic dissimilarity of non-cyanobacterial communities between two groups of lakes. STAMP was used to identify the bacterial families, which differed significantly between the two groups of lakes (Parks *et al*., 2014). Correlations between the relative abundance of each of the different families and that of combined methanotrophic lineages were further performed in the R statistical environment.

Data availability

Raw reads in this study have been deposited into the NCBI Sequence Read Archive (SRA) with the Project number PRJNA663707 (under the accession numbers SRR12649448-SRR12649525), and PRJNA575023 (under the accession numbers SRR10903090-SRR10903098).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting information.