# Title

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# Abstract

# Introduction

Freshwater lakes are important to global nutrient cycling. Lakes act as integrators of their surrounding landscapes, collecting nutrients from terrestrial ecosystems. This makes lakes “hotspots” in the landscape, particularly in carbon cycling. The contributions to nutrient cycling by lakes are significant on a global scale and are likely to change under warming conditions, making the study of freshwater nutrient cycling an important area of research.

Nutrient cycling in lakes has been previously thought of in terms of primary production vs. respiration, allochthonous vs autochthonous carbon, dissolved vs particulate matter, and organic vs. inorganic nutrients (McGowan et al. 2016). These broad categories are used to calculate budgets for lakes. However, microbes are responsible for most nutrient cycling in lakes, and freshwater microbes are diverse in both taxonomy and function. One of the grand challenges in freshwater microbial ecology is to link taxonomic groups to ecosystem functions in order to improve predictive models and budgets of lake nutrient cycling. To reach this level of understanding, the broad categories of nutrient cycling must be broken down into microbe-specific categories.

Because the vast majority of freshwater microbes cannot yet be cultured, sequencing data can be used instead to infer the function of specific taxonomic groups. Metagenomics has previously been used to shed light into the role of aquatic microbes. For example, this type of study has been used to investigate functional differences between salt and freshwater microbes (Eiler et al. 2015), to identify important functions in humic lakes (Peura et al. 2012), and to compare microbial communities on a global scale (Gimmler et al. 2016). However, many metagenomics-based studies take a gene-centric approach to investigating microbial functions. In this study, we use metagenome-assembled genomes (MAGs) to compare microbial functions between two lakes of different trophic statuses with an organism-centric approach instead. Analyzing genomes rather than genes provides better insight into the ecological roles of specific microbes within freshwater communities.

We hypothesized that we would be able to infer information about ecosystem-level functions based on genome content in our comprehensive dataset. This includes differences in primary production between lakes, preferences for degradation of autochthonous or allochthonous carbon, and biases towards certain steps in the nitrogen and sulfur cycles based on the availability of these inorganic compounds. Our analysis of 205 MAGs from Lake Mendota, a highly productive eutrophic lake, and Trout Bog, a humic or dystrophic bog lake, demonstrated many similarities in microbial functioning across ecosystems and confirmed previous research on freshwater microbes, but also revealed key differences based on the metabolisms predicted by our genomes.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Lake Mendota | Trout Bog Epilimnion | Trout Bog Hypolimnion |
| Location | Madison, WI | Boulder Junction, WI | Boulder Junction, WI |
| Depth of lake (m) | 25.3 | 7.9 | 7.9 |
| Surface area of lake (km2) | 39.61 | 0.01 | 0.01 |
| Microbial sampling range (m depth) | 0-12 | 0-2 | 2-7 |
| pH | 8.60 (0.35) | 5.00 (0.19) | 5.19 (0.24) |
| DIC (ppm) | 40.68 (6.67) | 1.79 (1.52) | 6.16 (5.51) |
| DOC (ppm) | 5.49 (0.92) | 18.10 (2.80) | 24.20 (5.51) |
| Total dissolved nitrogen (ppb) | 1070.38 (421.01) | 612.14 (153.12) | 1448.99 (1127.77) |
| Total nitrogen (ppb) | 1262.25 (353.04) | 754.45 (229.14) | 1711.86 (1509.75) |
| Total dissolved phosphorus (ppb) | 88.56 (57.53) | 13.45 (7.63) | 78.14 (95.81) |
| Total phosphorus (ppb) | 111.94 (47.11) | 27.12 (16.34) | 107.67 (122.66) |
| Chloride (ppm) | 39.87 (7.44) | 0.22 (0.07) | 0.28 (0.08) |
| Sulfate (ppm) | 18.04 (3.16) | 1.22 (0.34) | 0.84 (0.60) |
| Calcium (ppm) | 31.90 (5.04) | 1.38 (0.24) | 1.84 (0.35) |
| Magnesium (ppm) | 32.93 (2.72) | 0.39 (0.06) | 0.45 (0.06) |
| Sodium (ppm) | 19.48 (1.69) | 0.22 (0.07) | 0.25 (0.06) |
| Potassium (ppm) | 3.27 (0.28) | 0.64 (0.17) | 0.75 (0.17) |
| Iron (ppm) | 0.00 (0.01) | 0.31 (0.10) | 0.47 (0.09) |
| Manganese (ppm) | 0.00 (0.01) | 0.08 (0.07) | 0.09 (0.11) |

**Table 1. Characteristics of Lake Mendota and Trout Bog.** Trout Bog and Lake Mendota, two North Temperate Lakes - Long Term Ecological Research (NTL-LTER) sites, were chosen for this analysis due to their extensive environmental data and contrasting chemistry. The epilimnion of Lake Mendota and both layers of Trout Bog were sampled using an integrated water column for microbial DNA weekly during the ice-free periods in 2005, 2007, 2008, and 2009. Chemistry data was measured by NTL-LTER from depth discrete samples taken from 0 and 4 m for Lake Mendota, 0 m for the Trout Bog Epilimnion, and 3 and 7 m for the Trout Bog Hypolimnion. Values reported here are the means of all measurements in the sampling time span (2005 – 2009), with standard deviations reported in parentheses.

# Results/Discussion

## Overview of Dataset

Analyzing the genomes of uncultured microbes can provide insight into the potential metabolic functions of those organisms. 205 bacterial metagenome assembled genomes (MAGs) were recovered from a metagenomic time series in Trout Bog and Lake Mendota as described in Bendall, et al (Bendall et al. 2016). These MAGs range in completeness from 50 to 99% complete, and passed quality checks for contamination using CheckM (Parks et al. 2015) <Table S1, MAG\_information.csv>. Of the 204 MAGs, 101 were recovered from Lake Mendota, 31 were recovered from the epilimnion of Trout Bog, and 69 were recovered from the hypolimnion of Trout Bog. Several MAGs in the epilimnion and hypolimnion of Trout Bog appeared to be from the same population based on high average nucleotide identities <Table S1, MAG\_information.csv>. The phylogenetic distribution of MAGs was consistent with the classifications of 16S ribosomal rRNA gene amplicon sequencing results (Figure 1). These results are consistent with other 16S-based studies in these sites (Hall et al. 2017; Linz et al. 2017).

<Fig 1a Barchart of MAG phyla>

<Fig 1b Barchart of 16S phyla>

**Figure 1. How representative are the MAGs of the microbial community?** The taxonomic classifications of MAGs (A) reflect the community composition observed via 16S rRNA ribosomal amplicon sequencing (B).

## Photosynthesis and Carbon Fixation

Primary production is a critical component of the carbon cycle in lakes. Therefore, we looked at potential routes of primary production within the microbial community, expecting to find differences between our two ecosystems. In Lake Mendota, MAGs classified as Cyanobacteria comprised the majority of photoautotrophs in the dataset. These populations contained genes encoding enzymes in the Calvin-Benson-Bassham (CBB) pathway. In Trout Bog, genomes appearing to be from photoautotrophic organisms were classified as *Chlorobium clathratiforme*, a species of Chlorobiales widespread in humic lakes (Karhunen et al. 2013). The Chlorobiales MAGs in Trout Bog contained genes encoding citrate lyase and other key enzymes in the reductive TCA cycle, an alternative carbon fixation method to the CBB pathway commonly found in green sulfur bacteria (Kanao et al. 2002; Tang and Blankenship 2010). The primary producers Cyanobacteria and Chlorobiales seem to perform similar ecosystem functions, such as nitrogen and carbon fixation, in their respective lakes; however, oxygen availability drives both the type of microbe acting in this role and the pathways that it uses for primary production.

Marker genes for anoxygenic photosynthesis were identified in several other MAGs of Burkholderiales from both lakes (classified as groups such as *Polynucleobacter necessarius*, *Lautropia*, and *Albidoferax*) (Martinez-Garcia et al. 2012). However, genes potentially encoding carbon fixation were identified in only one of these MAGs, sequenced from Lake Mendota and classified as Burkholderiales.

Another form of harvesting sunlight for energy in freshwater is the use of light-activated proteins such as rhodopsins. Rhodopsins were observed in many phylogenetically diverse MAGs in both Trout Bog and Lake Mendota, and have been the subject of further study (cite Shaomei’s preprint when it comes out).

## Carbon Degradation

Carbon in lakes can either be produced in the water column (autochthonous) or received from the surrounding terrestrial landscape (allochthonous). To further understand bacterial carbon degradation in lakes, we identified and categorized putative carbon degradation pathways in our MAGs. We hoped to discern between allochthonous and autochthonous carbon degradation, as this is a common approach to investigating carbon cycling in limnology. However, because both kinds of carbon contain similar moieties or undergo transformations making the origin of the carbon difficult to trace, we could not distinguish between allocthonous and autochthonous degradation pathways based on genome content. Instead, we divide carbon degradation pathways into three main types observed in our genomes: biopolymer degradation, carbohydrate degradation, and methylotrophy.

Biopolymers in freshwater can be allochthonous or autochthonous in origin. Two common biopolymers, cellulose and chitin, are produce on land and in the water column, respectively. In both lakes, Bacteroidetes and Verrucomicrobia MAGs contained genes encoding cellulases, chitinases, and glucoside hydralases, without appearing to specialize in autochthonous or allochthonous carbon. However, a study of these Verrucomicrobia MAGs found that the profiles of glucoside hydralases differed between Lake Mendota and Trout Bog, potentially reflecting the differences in autochthonous and allochthonous carbon sources (He et al. 2017). Glucoside hydralase – encoding genes were also identified in Planctomycetes in Lake Mendota and in Burkholderiales, Actinobacteria, and Methylococcales in Trout Bog. Genes relating to the degradation of cellobiose and chitobiose, breakdown products of cellulose and chitin, were common in many taxa from both lakes. Degradation of phenol and salicylate, two aromatic compounds derived from terrestrial carbon sources, were potentially identified in MAGs of Burkholderiales in both lakes.

Although eukaryotic genomes were not included in this analysis, eukaryotic algae are known photoautotrophs in both lakes (Descy et al. 2000; Hurley and Armstrong 1990) Algae produce amino acids, carbohydrates, and carboxylic acids that fuel growth of the heterotrophic community (Salcher, Posch, and Pernthaler 2013). We observed many MAGs in both Lake Mendota and Trout Bog containing putative pathways for the degradation of carbohydrates such as glucose, galactose, maltose, rhamnose, mannose, and xylose. These compounds are all documented algal exudates in freshwater (Giroldo, Augusto, and Vieira 2005; Juttner and Matuschek 1977). They can also be derived from the breakdown of biopolymers; leaky extracellular degradation of biopolymers may result in these sugars being made available to community members without the ability to break down biopolymers. Degradation of additional sugars involved in galactose metabolism (sucrose, stachyose, raffinose, trehalose, lactose, and melibiose) were identified in MAGs classified as Bacteroidetes, Verrucomicrobia, and Actinobacteria from Trout Bog, but not from Lake Mendota.

Methylotrophy, the ability to grow solely on one carbon compounds such as methane, methanol, formaldehyde, or methylamines, were identified in Trout Bog and Lake Mendota. Multiple MAGs classified as well-studied methylotrophs Methylococcales and Methylophilaceae contained genes for methylotrophic pathways in both lakes (Figure 3). While many of the sequenced methylotrophs in freshwater are derived from sediment, they have also been identified in the water column (Kalyuzhnaya et al. 2012; Salcher et al. 2015). Although pelagic methylotrophs may potentially have different traits than sedimentary methylotrophs, the genome content of our MAGs suggested that in terms of carbon degradation, these bacteria are highly similar. Additionally, Lake Mendota contained MAGs containing potential methylotrophs belonging to Planctomyces and Rhodocyclaceae, while additional potential methylotrophs in Trout Bog included Burkholderiales, Rhizobiales, Nitrosomonadales, Geobacteraceae, and Solirubrobacterales. Given the rapid rate at which the known diversity of methylotrophs is increasing, this finding is not surprising (Chistoserdova, Kalyuzhnaya, and Lidstrom 2009). The methylotrophs Methylobacter and Methylotenera have been observed to exchange carbon cooperatively, perhaps outcompeting other community members via denitrification (Beck et al. 2013). Methylotrophy appears to be an important step in the freshwater carbon cycle in Lake Mendota and Trout Bog, where it may be a link between carbon cycling and other nutrients.

A screenshot of a cell phone

Description generated with high confidence

**Fig 2. Carbon cycling in Lake Mendota vs Trout Bog.** Carbon cycling between the two lakes was largely similar, with a few key differences. Carbon fixation is carried out by different taxa with different pathways. MAGs from Trout Bog tend to over more genes encoding enzymes in galactose metabolism than those from Lake Mendota.

**A close up of a map

Description generated with high confidenceFigure 3. Methylotrophy in freshwater.** *Methylococcaceae* and *Methylotenera* are taxa capable of methylotrophy in both Lake Mendota and Trout Bog. While both are likely capable of subsisting only on one carbon compounds, both have the potential to utilize other carbon sources. This, along with their putative pathways for assimilatory sulfate reduction and Methylococcaceae’s potential for denitrification and nitrogen fixation, place these populations at the intersection of multiple nutrient cycles.

## Carbohydrate Active Enzymes

Shaomei’s Verruco paper suggests we’ll see far more glucoside hydrolases in Trout Bog than in Lake Mendota. Planctomyces in Lake Mendota had many GHs, though. Move that here and eliminate unusual microbes section?

Include figure of heatmap of GH families by order by lake

## Nitrogen Cycling

While carbon cycling was relatively similar between lakes, the drastically different concentrations of nitrogen in Trout Bog versus Mendota led us to hypothesize that steps in the water column nitrogen cycle may be altered between these two systems. One key difference we found in the MAGs was that in Mendota, very few MAGs had genes encoding nitrogen fixation, and they belong mainly to Cyanobacteria. Conversely, more MAGs in Trout Bog contained these genes, and they were in phylogenetically diverse populations. The fact that an ecologically important function, nitrogen fixation, is carried out by a single phylogenetic group in Lake Mendota, implies that Cyanobacteria have a disproportionate impact on the nitrogen cycle in this ecosystem. This may also be a factor in the documented links between cyanobacterial bloom toxicity and nitrogen fixation in Lake Mendota (Beversdorf, Miller, and McMahon 2013). Genes annotated as nitrate and nitrite reductases, key enzymes in denitrification, were found in MAGs from both lakes. However, nitrate reductases were far less common than nitrite reductases in Lake Mendota (19 vs 53, respectively), and found primarily in Cyanobacteria. This difference was not as pronounced in Trout Bog, although the reasons for this trend are unknown. Genes annotated as ammonia monooxygenase were not found in MAGs from either lake, aside from the ammonia/methane monooxygenases found in MAGs classified as putative methanotroph Methylococcales, which are likely not involved in nitrogen cycling. No genes potentially encoding the anammox pathway were identified any of the MAGs. Genes encoding steps in the urea cycle or ammonia assimilation were found in nearly every MAG. This is consistent with previous research, where urea was found to be a significant nitrogen source for freshwater bacteria, particularly in epilimnia (Jorgenson et al. 1998), and where algae and bacteria were observed to compete for urea in an estuarine system (Remsen, Carpenter, and Schroeder 1972).

Genes potentially encoding the biosynthesis, degradation, and transport of the polyamines and non-proteinogenic amino acids putrescine, spermidine, and canavanine were widespread in both lakes. While there is some evidence for the importance of polyamines in aquatic systems (Mou et al. 2011), these compounds have been less studied in freshwater and their ecological role is not yet resolved. Our results lend support to the hypothesis that these compounds are important parts of the dissolved organic nitrogen pool in freshwater. Polyamines play a critical but poorly understood role in bacterial metabolism (Igarashi and Kashiwagi 1999), and the exchange of these nitrogen compounds may be a factor structuring freshwater bacterial communities. One likely source of polyamines is higher trophic levels such as fish or zooplankton, as these compounds can result from the decomposition of amino acids.

A screenshot of a cell phone

Description generated with very high confidence

**Fig 3A. Nitrogen cycling in Trout Bog vs Lake Mendota.** The numbers of genes relating to nitrogen cycling in the MAGs were analyzed by lake. Genes annotated as nitrogenase subunits were more common in Trout Bog than Lake Mendota. Nitrite reductases were observed more frequently than nitrate reductases in both lakes, but this difference was more pronounced in Lake **A close up of a map

Description generated with high confidence**Mendota. Polyamine transport was widespread in both lakes.

**Fig 3B. Nitrogen cycling in Trout Bog vs Lake Mendota.** The potential to fix nitrogen was confirmed in MAGs by searching for operons containing multiple nitrogen fixation – related genes. Trout Bog had greater numbers of MAGs that met this condition than Lake Mendota. The putative nitrogen fixers in Lake Mendota were all Cyanobacteria, while putative nitrogen fixers in Trout Bog were more diverse.

## Sulfur Cycling

Sulfur is another important element in freshwater. Because measurements of sulfur species measurements were not available for either lake, we did not predict any differences in sulfur cycling between lakes. We found that, as with carbon cycling, the identity and numbers of genes associated with steps in the sulfur cycle were similar between Lake Mendota and Trout Bog. Sulfate reduction genes dominated over sulfide oxidation genes in both systems, presumably reflecting the ecological importance of oxidation vs reduction. Genes potentially encoding assimilatory sulfate reduction were far more common than those potentially encoding dissimilatory sulfate reduction. This indicates that sulfate is likely more often used as a building block in biosynthesis rather than as a terminal electron acceptor. Genes for sulfur cycling were identified in diverse MAGs in both lakes.

A screenshot of a cell phone

Description generated with high confidence

**Fig 4. Number of MAGs containing sulfur metabolism genes by lake.** Sulfate reduction dominates over sulfide oxidation in both ecosystems. By the number of MAGs encoding putative enzymes for each pathway, assimilatory sulfate reduction is favored over dissimilatory sulfate reduction. These results likely reflect the availability of sulfur species in Trout Bog and Lake Mendota.

## Unusual microbes

Although our primary goal was to use genome content to investigate differences in nutrient cycling between lakes, we recovered the genomes of unusual microorganisms in this dataset and report their genome content here. One MAG from Elusimicrobiales (formerly Termite Group 1) was recovered from Trout Bog. While this genome is only 44% complete, we can propose that it uses sugars such as maltooligosaccharides, maltose, and arabinogalactan as a carbon source. This population of Elusimicrobiales likely reduces sulfate via the assimilatory pathway. It also contains one nitrogenase subunit, suggesting that it may be capable of fixing nitrogen. The abilities to fix nitrogen and degrade saccharides suggested by our freshwater MAG are consistent with the physiology of cultured representatives of Elusimicrobia isolated from insect guts (D P R Herlemann et al. 2009; Zheng et al. 2016). Although Elusimicrobia have not been previously studied in freshwater, Elusimicrobia are thought to be more diverse and widespread in the environment than previously assumed (Daniel P R Herlemann, Geissinger, and Brune 2007).

Thirteen MAGs classified as Planctomycetes were recovered from Lake Mendota. While some Planctomycetes isolates have been known to perform anammox, no genes encoding the enzymes necessary for this pathway were found in the Lake Mendota MAGs. The Mendota Plantcomycetes MAGs contained genes encoding glucoside hydrolases and sulfatase enzymes. These pathways could potentially be used to break down complex sulfur-containing polysaccharides, such as those produced by Cyanobacteria or eukaryotic algae. Similar genes were found in Planctomycetes genomes in a freshwater algal biofilm (cite when published). This suggests a new potential ecological role for Planctomycetes in freshwater as a motile specialist in polysaccharide degradation.

A picture containing text, map

Description generated with very high confidence

**Figure 5. Proposed metabolisms of Planctomycetes in Lake Mendota.** Several MAGs classified as Planctomycetes were recovered from Lake Mendota. The observation of genes annotated as glycoside hydrolases and sulfatases suggest a role for Planctomycetes as algal polysaccharide degraders. These populations also appear to consume saccharides such as galactose, fructose, and mannose, and synthesize both spermidine and putrescine. The presence of genes encoding flagellar and chemotaxis proteins suggests that they are motile.

## Add section on # read mapping back to each MAG across the time series

This is something Shaomei’s reviewers wanted. Don’t need an in depth analysis – just classify as abundant vs rare, persistent vs variable. Will map as soon as Gluster on CHTC is back up and running.

Use SQUID instead if file sizes are below 1GB?

## Conclusions

Analysis of gene content and predicted pathways in our MAGs reveals potential similarities and differences in the ecology of bacteria in two freshwater lakes. Surprisingly, predicted pathways involved in carbon cycling were often similar between Lake Mendota and Trout Bog. However, exceptions included differences in the identity and carbon fixation pathways of primary producers, and the greater numbers of genes encoding enzymes involved in galactose metabolism in Trout Bog. Sulfur cycling was also highly similar between these two lakes, with both indicating a bias towards reduction over oxidation, and furthermore towards assimilatory over dissimilatory reduction. Nitrogen cycling did appear altered between the lakes, with the greater levels of nitrogen fixation (and diversity of nitrogen fixers) in Trout Bog compared to Lake Mendota in concordance with their known nitrogen concentrations. Polyamines and nonproteinogenic amino acids appear to be important sources of nitrogen in both lakes. Finally, comprehensive analysis of individual MAGs can suggest ecological roles for uncultured organisms, such as polysaccharide degradation for Lake Mendota’s Planctomycetes, saccharide degradation for Trout Bog’s Elusimicrobiales, and a key position at the intersection of carbon, nitrogen, and sulfur cycling for methylotrophs in both lakes.

The insights gained from this study provide hypotheses for further testing of bacterial nutrient cycling in freshwater through metatranscriptomics and chemical assays. Additionally, genomes from this study can be compared to those sequenced from other lakes and environments. We hope that this will serve as a resource to other researchers with similar genomes and datasets.

# References

Beck, David a.C. et al. 2013. “A Metagenomic Insight into Freshwater Methane-Utilizing Communities and Evidence for Cooperation between the Methylococcaceae and the Methylophilaceae.” *PeerJ* 1: e23. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3628875&tool=pmcentrez&rendertype=abstract.

Bendall, Matthew L et al. 2016. “Genome-Wide Selective Sweeps and Gene-Specific Sweeps in Natural Bacterial Populations.” *ISME journal* 10: 1589–1601. http://dx.doi.org/10.1038/ismej.2015.241.

Beversdorf, Lucas J, Todd R Miller, and Katherine D McMahon. 2013. “The Role of Nitrogen Fixation in Cyanobacterial Bloom Toxicity in a Temperate , Eutrophic Lake.” *PloS one* 8(2): 1–11.

Chistoserdova, Ludmila, Marina G. Kalyuzhnaya, and Mary E. Lidstrom. 2009. “The Expanding World of Methylotrophic Metabolism.” *Annual review of microbiology* 63: 477–99.

Descy, Jean-Pierre et al. 2000. “PIGMENT RATIOS AND PHYTOPLANKTON ASSESSMENT IN NORTHERN WISCONSIN LAKES.” *Journal of Phycology* 36: 274–86.

Eiler, Alexander et al. 2015. “Tuning Fresh: Radiation through Rewiring of Central Metabolism in Streamlined Bacteria.” *The ISME Journal* (January 2016): 1–13. http://dx.doi.org/10.1038/ismej.2015.260.

Gimmler, Anna et al. 2016. “The Tara Oceans Voyage Reveals Global Diversity and Distribution Patterns of Marine Planktonic Ciliates.” *Nature Publishing Group* (April): 1–13. http://dx.doi.org/10.1038/srep33555.

Giroldo, Danilo, Armando Augusto, and Henriques Vieira. 2005. “Polymeric and Free Sugars Released by Three Phytoplanktonic Species from a Freshwater Tropical Eutrophic Reservoir.” *Journal of Plankton Research* 27(7): 695–705.

Hall, Michael W et al. 2017. “Ananke : Temporal Clustering Reveals Ecological Dynamics of Microbial Communities.” *PeerJ* 5(e3812): 1–19.

He, Shaomei et al. 2017. “Ecophysiology of Freshwater Verrucomicrobia Inferred from Metagenome-Assembled Genomes.” *mSphere* 2(5): 1–17.

Herlemann, Daniel P R, Oliver Geissinger, and Andreas Brune. 2007. “The Termite Group I Phylum Is Highly Diverse and Widespread in the Environment.” *Applied and Environmental Microbiology* 73(20): 6682–85.

Herlemann, D P R et al. 2009. “Genomic Analysis of ‘ Elusimicrobium Minutum ,’ the First Cultivated Representative of the Phylum ‘ Elusimicrobia ’ ( Formerly Termite Group 1 ).” *Applied and Environmental Microbiology* 75(9): 2841–49.

Hurley, James P, and David E Armstrong. 1990. “Fluxes and Transformations of Aquatic Pigments in Lake Mendota , Wisconsin.” *Limnology and Oceanography* 35(2): 384–98.

Igarashi, Kazuei, and Keiko Kashiwagi. 1999. “Polyamine Transport in Bacteria and Yeast.” *Biochem. J.* 344: 633–42.

Jorgenson, Niels OG et al. 1998. “Effects of Sunlight on Occurrence and Bacterial Turnover of Specific Carbon and Nitrogen Compounds in Lake Water.” *FEMS Microbiology Ecology* 25: 217–27.

Juttner, F, and T Matuschek. 1977. “The Release of Low Molecular Weight Compounds by the Phytoplankton in an Eutrophic Lake.” *Water* 12: 251–55.

Kalyuzhnaya, Marina G et al. 2012. “Novel Methylotrophic Isolates from Lake Sediment, Description of Methylotenera Versatilis Sp. Nov. and Emended Description of the Genus Methylotenera.” *International journal of systematic and evolutionary microbiology* 62(Pt 1): 106–11. http://www.ncbi.nlm.nih.gov/pubmed/21335496.

Kanao, Tadayoshi et al. 2002. “Characterization of Isocitrate Dehydrogenase from the Green Sulfur Bacterium Chlorobium Limicola: A Carbon Dioxide-Fixing Enzyme in the Reductive Tricarboxylic Acid Cycle.” *European Journal of Biochemistry* 269(7): 1926–31.

Karhunen, Jatta, Lauri Arvola, Sari Peura, and Marja Tiirola. 2013. “Green Sulphur Bacteria as a Component of the Photosynthetic Plankton Community in Small Dimictic Humic Lakes with an Anoxic Hypolimnion.” *Aquatic Microbial Ecology* 68: 267–72.

Linz, Alexandra M. et al. 2017. “Bacterial Community Composition and Dynamics Spanning Five Years in Freshwater Bog Lakes.” *mSphere* 2(3): 1–15.

Martinez-Garcia, Manuel et al. 2012. “High-Throughput Single-Cell Sequencing Identifies Photoheterotrophs and Chemoautotrophs in Freshwater Bacterioplankton.” *The ISME Journal* 6(1): 113–23. http://dx.doi.org/10.1038/ismej.2011.84.

McGowan, Suzanne et al. 2016. “Long-Term Perspectives on Terrestrial and Aquatic Carbon Cycling from Palaeolimnology.” *WIREs Water* 3: 211–34.

Mou, Xiaozhen et al. 2011. “Metatranscriptomic Signature of Exogenous Polyamine Utilization by Coastal Bacterioplankton.” 3: 798–806.

Parks, Donovan H et al. 2015. “CheckM: Assessing the Quality of Microbial Genomes Recovered from Isolates, Single Cells, and Metagenomes.” *Genome Research* 25(7).

Peura, Sari et al. 2012. “Distinct and Diverse Anaerobic Bacterial Communities in Boreal Lakes Dominated by Candidate Division OD1.” *ISME journal* 6: 1640–52.

Remsen, Charles C, Edward J Carpenter, and Brian W Schroeder. 1972. “Competition for Urea among Estuarine Microorganisms.” *Ecological Society of America* 53(5): 921–26.

Salcher, Michaela M, Stefan M Neuenschwander, Thomas Posch, and Jakob Pernthaler. 2015. “The Ecology of Pelagic Freshwater Methylotrophs Assessed by a High-Resolution Monitoring and Isolation Campaign.” *The ISME Journal*: 1–12. http://www.nature.com/doifinder/10.1038/ismej.2015.55.

Salcher, Michaela M, Thomas Posch, and Jakob Pernthaler. 2013. “In Situ Substrate Preferences of Abundant Bacterioplankton Populations in a Prealpine Freshwater Lake.” *Isme J* 7(5): 896–907. http://dx.doi.org/10.1038/ismej.2012.162 (November 15, 2013).

Tang, Kuo Hsiang, and Robert E. Blankenship. 2010. “Both Forward and Reverse TCA Cycles Operate in Green Sulfur Bacteria.” *Journal of Biological Chemistry* 285(46): 35848–54.

Zheng, Hao, Carsten Dietrich, Renate Radek, and Andreas Brune. 2016. “Endomicrobium Proavitum , the First Isolate of Endomicrobia Class . Nov . ( Phylum Elusimicrobia ) – an Ultramicrobacterium with an Unusual Cell Cycle That Fixes Nitrogen with a Group IV Nitrogenase.” *Environmental Microbiology* 18(1): 191–204.