# 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 nearly 200 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. 194 high quality 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 194 MAGs, 100 were recovered from Lake Mendota, 31 were recovered from the epilimnion of Trout Bog, and 63 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 S2, ANI\_matrix.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

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). In addition to genes suggesting the presence of the CBB pathway, 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 commonly found in green sulfur bacteria (Kanao et al. 2002; Tang and Blankenship 2010). Both photoautrophs contained genes potentially encoding nitrogen fixation as well. The primary producers Cyanobacteria and Chlorobiales seem to perform similar ecosystem functions 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.

The potential for photoheterotrophy via anoxygenic aerobic photosynthesis was identified in several MAGs from both lakes (Martinez-Garcia et al. 2012). Proteobacteria, particularly Burkholderiales, most often contained the marker genes for this process, although these genes were not shared broadly across the phylum. A MAG of Acidobacteria from the Trout Bog epilimnion also contained genes suggesting anoxygenic aerobic photosynthesis. Another form of photoheterotrophy previously identified in freshwater is the use of light-activated proteins such as rhodopsins. We observed genes encoding rhodopsins in MAGs from both lakes, but particularly in MAGs from Lake Mendota classified as Actinobacteria and Bacteroidetes. These MAGs and their potential rhodopsins are the subject of further study (cite Shaomei’s preprint when it comes out).

## Central Metabolism and Simple Carbon Degradation

Freshwater contains a variety of low-complexity carbon sources such as carbohydrates, carboxylic acids, and one-carbon compounds. While carbon in freshwater is often divided into autochthonous (originating within the lake) and allochthonous (derived from the surrounding landscape) carbon, this distinction is less clear for bacteria. For example, there is substantial overlap in algal exudates and cellulose breakdown products, and while one-carbon compounds such as methane are produced in the lake, they are often produced via the decomposition of allochthonous carbon. Therefore, we found it more informative to categorize the carbon degradation pathways observed in our dataset by carbon complexity (Fig. 2).

Central metabolism is often the entry point for the least complex carbon compounds, and central metabolic pathways may reveal how a bacterium is using a carbon compound. The tricarboxylic acid (TCA) cycle, arguably the most central pathway in bacteria, was notably absent in MAGs classified as Tenericutes in Lake Mendota and in unclassified MAGs in the hypolimnion of Trout Bog. Genes encoding enzymes in the glyoxylate cycle, a variant of the TCA cycle that is used to produce biosynthetic intermediates when glucose is not available, were observed in Chlamydiae in Lake Mendota, Acidobacteria in Trout Bog, and in some Proteobacteria in both lakes. The pentose phosphate pathway, both oxidative and non-oxidative phases, was found in MAGs from most phyla.

Sugars in freshwater can be derived either from algae or from the breakdown of terrestrial biopolymers (Giroldo, Augusto, and Vieira 2005; Juttner and Matuschek 1977). In our MAGs, genes encoding the pathway for mannose degradation appeared frequently in both lakes. Mannose feeds into glycolysis, and can be used as the sole source of carbon and energy in bacteria such as E. coli (cite from MetaCyc). Genes encoding the degradation of rhamnose and fucose, whose pathways converge to enter glycolysis and produce pyruvate, were frequently found within the same MAGs (including members of Planctomycetes and Verrucomicrobia in Lake Mendota, and members of Bacteroidetes, Ignavibacteria, and Verrucomicrobia in Trout Bog). Putative pathways for the degradation of galactose were often observed in these same MAGs. Xylose is a freshwater sugar which has already been identified as potential carbon source for streamlined Actinobacteria (cite); this was confirmed in our MAGs, with Bacteroidetes, Planctomycetes, and Verrucomicrobia in Lake Mendota and Bacteroidetes and Verrucomicrobia in Trout Bog as additional potential xylose degraders. Genes for the degradation of glycolate, a compound produced by algae (cite Paver), were identified in Cyanobacteria and Proteobacteria in Lake Mendota and in Acidobacteria, Proteobacteria, and Verrucomicrobia in Trout Bog.

Methylotrophy, the ability to grow solely on one carbon compounds such as methane or methanol, appears to be a likely metabolism in MAGs from both Trout Bog and Lake Mendota. Putative pathways for methanol degradation were found in MAGs classified as Methylophilales, while MAGs from Methylococcales were potential methane degraders. Methylotrophy in cultured freshwater isolates from these taxa is well-documented (Kalyuzhnaya et al. 2012; Salcher et al. 2015); however, genes encoding methanol degradation were also identified in MAGs from taxa not typically known as methylotrophs. These included MAGs classified as Burkholderiales, Rhizobiales, and Nitrosomonadales in Trout Bog. Given the rapid rate at which the known diversity of methylotrophs is increasing, this finding is intriguing, but not surprising (Chistoserdova, Kalyuzhnaya, and Lidstrom 2009).

## Complex Carbon Degradation

Biopolymers in freshwater can be autochthonous (ex. algal polysaccharides) or allocthonous (ex. cellulose). While degradation of these high-complexity carbon sources may require specialized enzymes, their wide availability and high yield of sugars make the ability to degrade complex carbon sources an advantageous trait. One way to analyze the ability to degrade high-complexity carbon is through genes annotated as glucoside hydrolases, enzymes that break bonds attached to carbohydrates. A previous study of Verrucomicrobia MAGs from our dataset found that the profiles of glucoside hydrolases differed between Lake Mendota and Trout Bog, potentially reflecting the differences in autochthonous and allochthonous carbon sources (He et al. 2017). We expanded this analysis of glucoside hydrolases to the entire dataset to identify differences in complex carbon degradation between lakes.

The coding density of glucoside hydrolases – the percentage of coding regions in a MAG annotated as a glucoside hydrolase – immediately revealed differences between Trout Bog and Lake Mendota, and even between the epilimnion and hypolimnion of Trout Bog (Fig. 3). The MAGs with the highest coding densities were found in members of Bacteroidales, Ignavibacteriales, Sphingobacteriales, and Verrucomicrobiales in the Trout Bog hypolimnion. The last two of those orders also contained MAGs with glucoside hydrolases in Lake Mendota and the Trout Bog epilimnion, but the others did not. There were several orders with glucoside hydrolases unique to Lake Mendota, including Mycoplasmatales (Tenericutes), Cytophagales (Bacteroidetes), Planctomycetales (Planctomycetes), and Puniceicoccales (Verrucomicrobia). In accordance with their ability to breakdown biopolymers to sugars, these MAGs from both lakes also contain putative degradation pathways for a variety of sugars (Fig. 2). The diversity of glucoside hydrolases, an indicator of the number of substrates an organism can degrade, correlated with density.

Several glucoside hydrolase families were abundant in Lake Mendota and in both layers of Trout Bog. Starting with the most abundant, these included GH109 (degrades cell walls and lipopolysaccharidies), GH74 (degrades glucose chains such as cellulose, starch, and glycogen), and GH23 (chitinase). While the most abundant glucoside hydrolases were similar between lakes, the increased diversity of these enzymes in Trout Bog’s hypolimnion suggested differences between their profiles of glucoside hydrolases. Lake Mendota contained unique glucoside hydrolases belonging to the family GH13 (specifically subfamilies 2, 5, and 21), which contain enzymes related to cellulose degradation. The only unique glucoside hydrolase in the Trout Bog epilimnion was GH62, a family of arabinofuranosidases. The hypolimnion contained many more unique enzymes than Lake Mendota or the epilimnion of Trout Bog, the most abundant of which were GH129 and GH89 (a-N-acetylgalactosaminidase), GH43\_12 (arabinanases, arabinofuranosidases, and xylosidases), GH44 (breakdown of long polysaccharides and oligomers), GH66 (dextranases and glucanotransferases), and GH67 (alpha-glucuronidase).

The increased density and diversity of glucoside hydrolases in the Trout Bog hypolimnion suggest that the bacterial community in this region relies more on complex carbon sources than simple carbon sources compared to Lake Mendota or even the epilimnion of Trout Bog. This may be because primary production results in increased availability of low complexity carbon compounds in epilimnia. However, the taxonomic profile of MAGs containing glucoside hydrolases differed by lake and layer, even when the profiles of glucoside hydrolases themselves were more similar.

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.

## Carbohydrate Active Enzymes

A close up of a piece of paper

Description generated with very high confidenceShaomei’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?

## Nitrogen Cycling

Nitrogen availability is an important factor structuring freshwater bacterial communities. Bog lakes such as Trout Bog are generally considered to be nitrogen-limited ecosystem, and what nitrogen is present is often bound in complex carbon compounds. Lake Mendota, as a eutrophic ecosystem, is considered to have excess nitrogen due to urban and agricultural pollution. However, this nitrogen is quickly incorporated into biomass and is not always readily available to microbes. Because of these different nitrogen regimes, we expected to see differences in the nitrogen metabolisms of Trout Bog vs Lake Mendota.

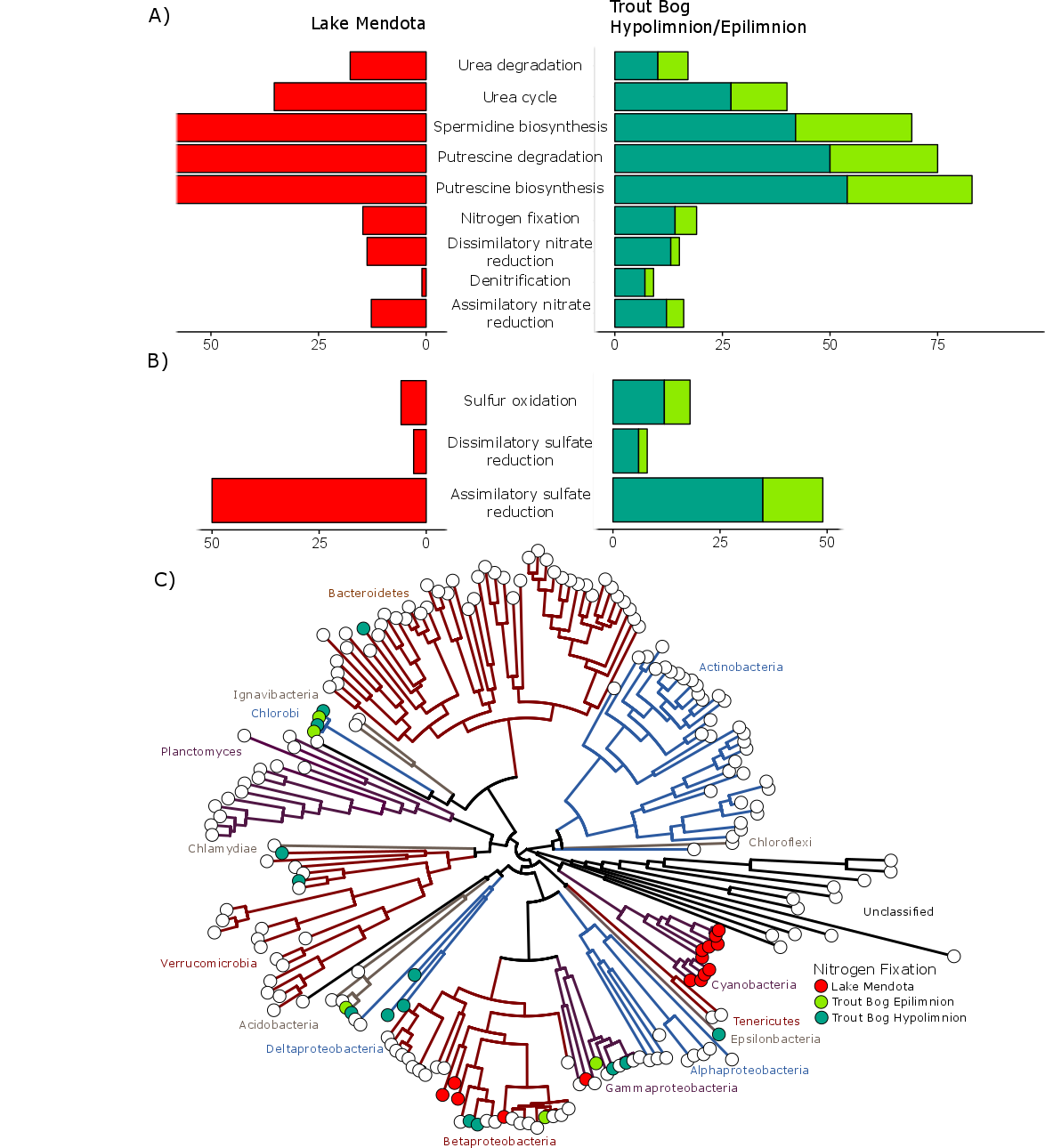
Properties of genomes themselves may provide information about nitrogen limitation. We observed a bias in MAGs from Trout Bog towards encoding amino acids with less nitrogen compared to MAGs from Lake Mendota using a Wilcoxon rank sum test (p = 0.02). This suggests that bacteria in Trout Bog have lower nitrogen requirements than bacteria in Lake Mendota. (How does this compare to marine systems? Cite Chisholm). GC content and estimated genome size, other potential indicators of nitrogen limitation, were not significantly different between lakes (p = 0.78 and p = 0.16, respectively). While amino acid bias suggests that limiting nitrogen concentrations in Trout Bog may lead to selection for organisms encoding nitrogen-poor proteins, other factors may be more important in determining properties such as genome size and GC content.

Putative pathways related to nitrogen metabolism appeared at similar frequencies from MAGs in both lakes. Dissimilatory and assimilatory nitrate reduction appear to be present in similar numbers of genomes, and denitrification appears slightly less often in genomes from both lakes. Urea degradation was predicted in MAGs of both lakes, consistent with 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).

We expected nitrogen fixation to be more prevalent in genomes from Trout Bog, but found instead that similar numbers of genomes contain genes encoding the potential pathway in Lake Mendota as well. However, taxonomy revealed differences between the two ecosystems. In Lake Mendota, nitrogen fixation appears restricted to Cyanobacteria and Betaproteobacteria. 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). MAGs containing genes encoding nitrogen fixation are more phylogenetically diverse in Trout Bog, including several classes of Proteobacteria, Verrucomicrobia, Chlorobi, and Bacteroidetes.

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.

Although we expected to find major differences in nitrogen metabolisms between lakes, we instead found striking similarities. Despite the chemical differences between lakes, similar nitrogen compounds are likely consumed by similar numbers of taxa. However, the identity of those taxa is the key difference between taxa, with nitrogen fixation as an example of a pathway with variable phylogenetic diversity between our two study sites.



**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 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 element structuring freshwater bacterial communities. 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.

**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.

## 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.