# Title

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# Abstract

# Introduction

Why nutrient cycling in lakes is important globally (lakes as integrators of the landscape, lakes as hotspots of carbon cycling, small lakes disproportionately active in carbon cycle, lakes as sentinels of change)

Description of study sites

How can metagenomic/genome analysis shed light on ecosystems?

Review other papers that have used this dataset (and why this paper is novel)

# 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) (supp table). Of the 205 MAGs, 102 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 (supp table). 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 2b 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.

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). Weobserved 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, as well as other low molecular weight compounds such as glycolate and citrate. These compounds are all documented algal exudates in freshwater (Giroldo, Augusto, and Vieira 2005; Juttner and Matuschek 1977).

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 will be the subject of further study (cite Shaomei’s preprint when it comes out).

## Degradation of Aquatic and Terrestrial Carbon

One important contrast in freshwater carbon cycling is the degradation of carbon produced in a lake (autocthonous) versus terrestrially-derived carbon (allocthonous). Because Lake Mendota and Trout Bog are surrounded by different landscapes, we expected to see differences in pathways for the degradation of allocthonous carbon but not in pathways for autochthonous carbon.

MAGs from multiply phyla in both lakes contained genes encoding pathways for the degradation of cellulose and aromatic compounds such as salicylate.

A type of autochthonous carbon degradation in freshwater is methylotrophy, the degradation of one carbon compounds such as methane, methanol, formaldehyde, or methylamines. Multiple MAGs classified as well-studied methylotrophs Methylococcales (an aerobic methanotroph) and Methylophilaceae (which likely degrades methanol and methylamines) containing genes for methylotrophic pathways were found in both lakes (Kalyuzhnaya et al. 2012; Salcher et al. 2015) (Figure 3). Consistent with studies of related cultured isolates, MAGs belonging to Methylococcales often contained operons encoding nitrogen fixation (Dedysh, Ricke, and Liesack 2004). Lake Mendota additionally had MAGs containing potential methylotrophs belonging to Planctomyces and Rhodocyclaceae, while additional potential methylotrophs in Trout Bog included Burkholderiales, Rhizobiales, Nitrosomonadales, Geobacteraceae, and Solirubrobacterales.

As previously mentioned, algal exudates are a major source of autochthonous carbon in freshwater. Genes encoding the pathways and transporters for degradation of amino acids and carbohydrates were widespread in MAGs from Lake Mendota and Trout Bog, as were genes encoding pathways for the degradation of chitin and its breakdown products, chitobiose and NAG.

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**Fig 2. Carbon degradation in Mendota vs Trout Bog.**

**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.

# A screenshot of a cell phone Description generated with very high confidenceNitrogen 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 was that in Mendota, very few MAGs had genes encoding nitrogen fixation, and they belong mainly to Cyanobacteria. Conversely, many MAGs in Trout Bog contained these genes, and they were in phylogenetically diverse populations. 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. Genes annotated as ammonia monooxygenase were not found in MAGs from either lake, aside from the ammonia/methane monooxygenases found in MAGs classified as the 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.

**A close up of a map

Description generated with high confidence**

**Fig 3. Nitrogen cycling in Trout Bog vs Mendota.** A) Number of marker genes for nitrogen cycling metabolisms in each lake B) Phylogeny of nitrogen fixers by lake

# Sulfur Cycling

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**Fig 4. Number of MAGs containing sulfur metabolism genes by lake**

Sulfur cycling is again relatively similar between Trout Bog and Lake Mendota in broad functions, if not the taxa responsible. Assimilatory sulfates reduction (where sulfates are incorporated into cell components) was more common than dissimilatory sulfate reduction (where sulfate is used as a terminal electron acceptor and sulfide is expelled outside the cell) in both systems. More pathways for sulfide oxidation were found in Trout Bog than in 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 micro-organisms in this process and report their genome content here. One MAG from Elusimicrobiales was recovered from Trout Bog. While this genome is only 44% complete, we can propose that uses sugars such as maltooligosaccharides, maltose, and arabinogalactan as a carbon source. This population of Elusimicrobiales reduces sulfate via the assimilatory pathway. It also contains one nitrogenase subunit, suggesting that it may be capable of fixing nitrogen.

Thirteen MAGs classified as Planctomycetes were recovered from Lake Mendota. A significant number of metagenomics reads in Lake Mendota were also classified as Planctomycetes, suggesting that this is an abundant group. While some Planctomycetes isolates have been known to perform anammox or degrade one-carbon compounds, no genes encoding the enzymes necessary for this pathway were found in the Lake Mendota MAGs. The gene content of a Planctomyces MAG from Trout Bog, however, suggested methylotrophy. The Plantcomycetes MAGs contained genes encoding glucoside hydrolases and sulfatase enzymes. These pathways could be used to break down complex polysaccharides, such as those produced by Cyanobacteria or eukaryotic algae. This suggests a new potential role for Planctomyces in freshwater as a specialist in polysaccharide degradation.

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**Figure 6B. Proposed functions of Planctomycetes in freshwater.** Observation of genes annotated as glycoside hydrolases and sulfatases suggest a role for Planctomycetes as polysaccharide degraders.

In conclusion…