

Here Comes the Bloom: Investigating Freshwater Cyanobacteria Bloom and Non-Bloom Sites in Ontario

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Abstract

Reports of algal blooms are on the rise both globally and domestically, more specifically here in Ontario. The number of blooms containing toxigenic cyanobacteria have been increasing, which poses major threats to the environment, as well as human and animal populations. Many models for studying blooms are able to predict biomass concentrations of cyanobacteria in large bodies of water, but there are a lack of models which are able to describe the composition and toxicity of these blooms.

Due to an increasing demand for the genetic evaluation of microbes associated with blooms, as well as the limitations involved with the culturing of cyanobacteria, a metagenomic approach was utilized in this study. This allowed for a deeper understanding of the microbial diversity of algal blooms, and permitted the assembly of potentially toxin producing genomes.

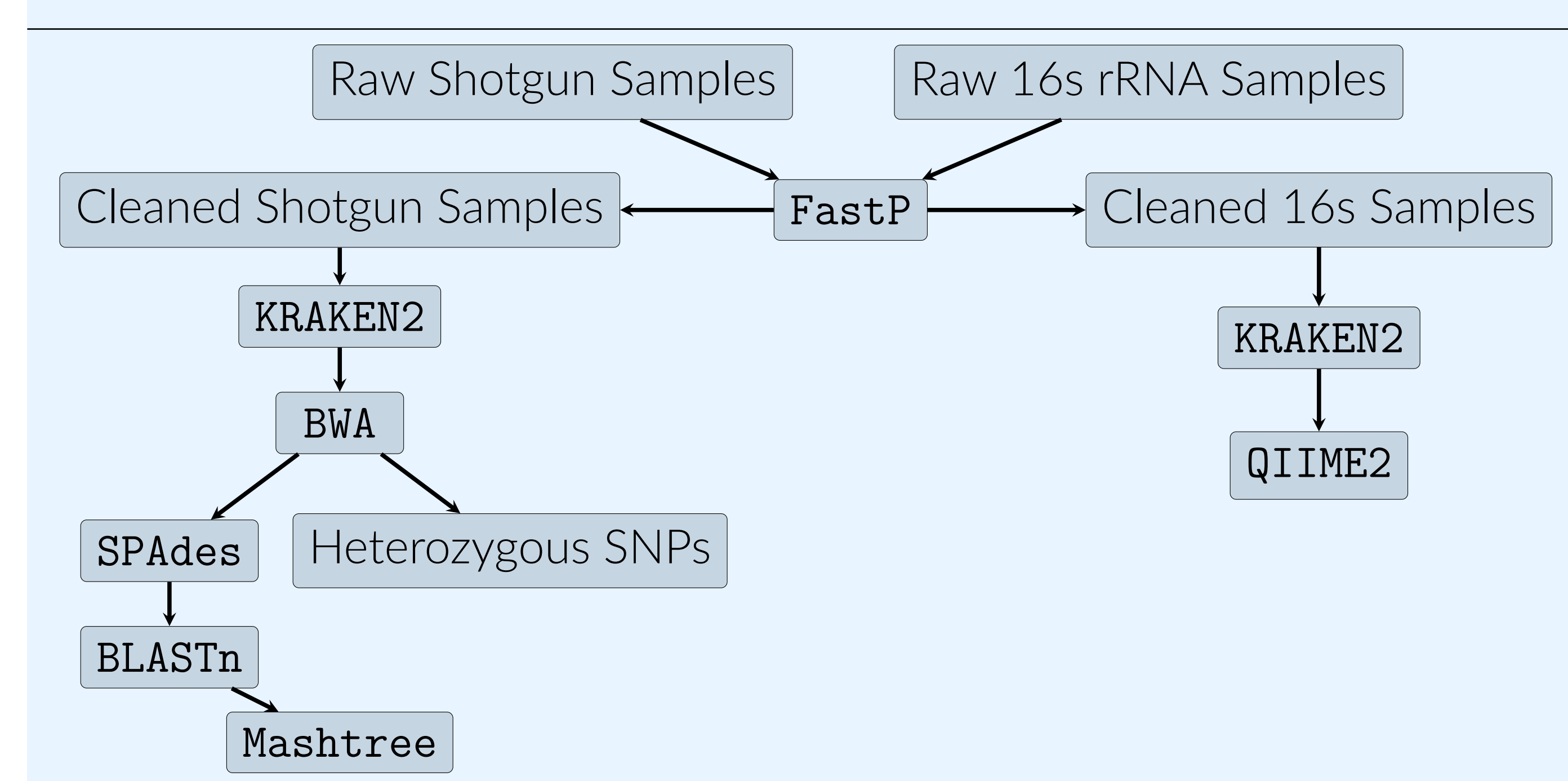
This study focuses on the composition of microbial communities in the collected samples along with the genomic assembly of four organisms found to play key roles in the development of harmful algal blooms. Overall, the findings reveal differences in cyanobacterial communities across bloom and non-bloom sites, as well as strain level differences in the organisms which dominate blooms and potentially contribute to toxicity.

Background

In collaboration with the Ministry of the Environment and Climate Change (MOECC), bloom and non-bloom sites were sampled from a variety of fresh-water locations around Ontario in 2015 and sent for 16s rRNA sequencing and shotgun metagenomic sequencing.

Type of Sequencing	Bloom Samples	Non-Bloom Samples	Unknown Samples
16s rRNA	16	4	36
Shotgun	12	16	0

Workflow



Phylogenetically Placing the Samples

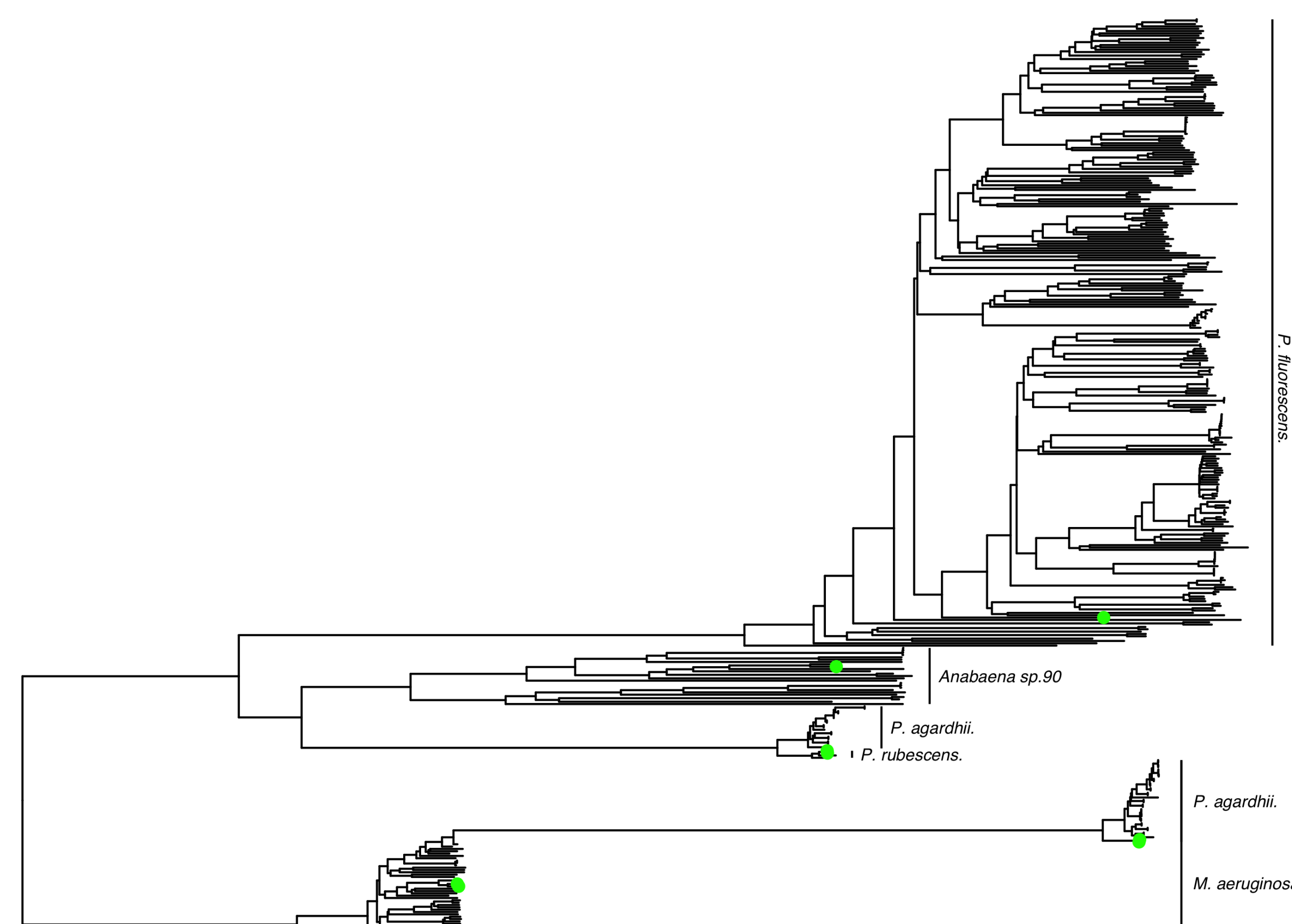


Figure 1. Mash Distance Phylogeny of five different species known to dominate algal blooms

Five different types of bacterial genomes were found to dominate in six bloom site shotgun samples. A Mash distance phylogeny was created which shows the blasted sample sequences among different strains of the same bacterial species collected from the NCBI database. The formation of unique clades is evident, and the blasted assemblies falling into the specific clades highlights the unique species of *Microcystis aeruginosa*, *Planktothrix rubescens*, *Planktothrix agardhii*, *Anabaena sp.90*, and *Pseudomonas aeruginosa* found in the samples.

Multiple Strain Potential at Bloom Sites

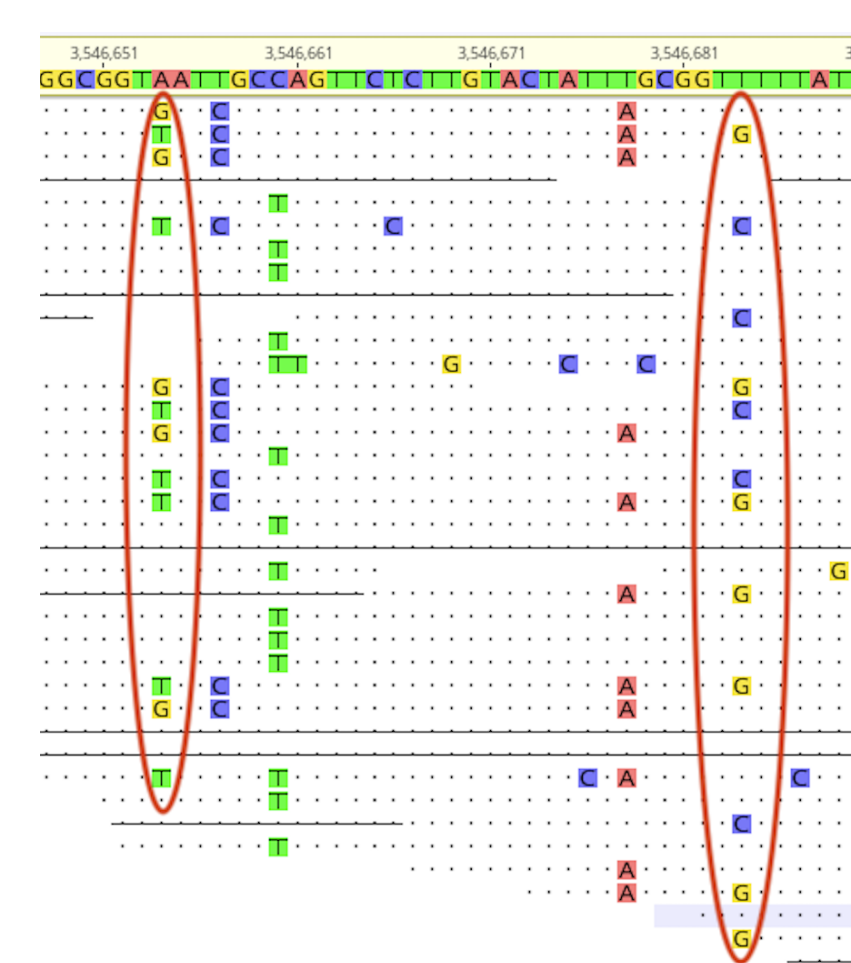


Figure 2. Showing two heterozygous SNP sites from a bloom sample containing *Microcystis aeruginosa*

Of the five different types of bacterial genera explored in the samples, four contained potentially toxic, microcystin producing strains. Single nucleotide polymorphisms (SNPs) were examined in bloom samples which contained these organisms in order to determine if heterozygous SNPs were present. Heterozygous SNPs were present in all four samples, with the largest amount being found in a sample containing *Microcystis aeruginosa*. These heterozygous SNPs provide potential evidence of different copies of the same gene and are therefore potentially indicative of multiple strains of *Microcystis aeruginosa* present at a single bloom site.

Differences in Bacterial Composition of Bloom and Non-Bloom Sites

Amplicon sequencing analysis using Quantitative Insights Into Microbial Ecology (QIIME2) revealed different bacterial compositions of bloom, non-bloom, and unknown sites.

- ⇒ At the phylum level, all types of samples tend to be dominated by proteobacteria, actinobacteria, and cyanobacteria.
- ⇒ Larger relative abundance of cyanobacteria in bloom sites compared to non-bloom sites, suggesting a key role played by cyanobacteria in the formation of blooms.
- ⇒ Larger relative abundance of verrucomicrobia in bloom sites compared to non-bloom sites.

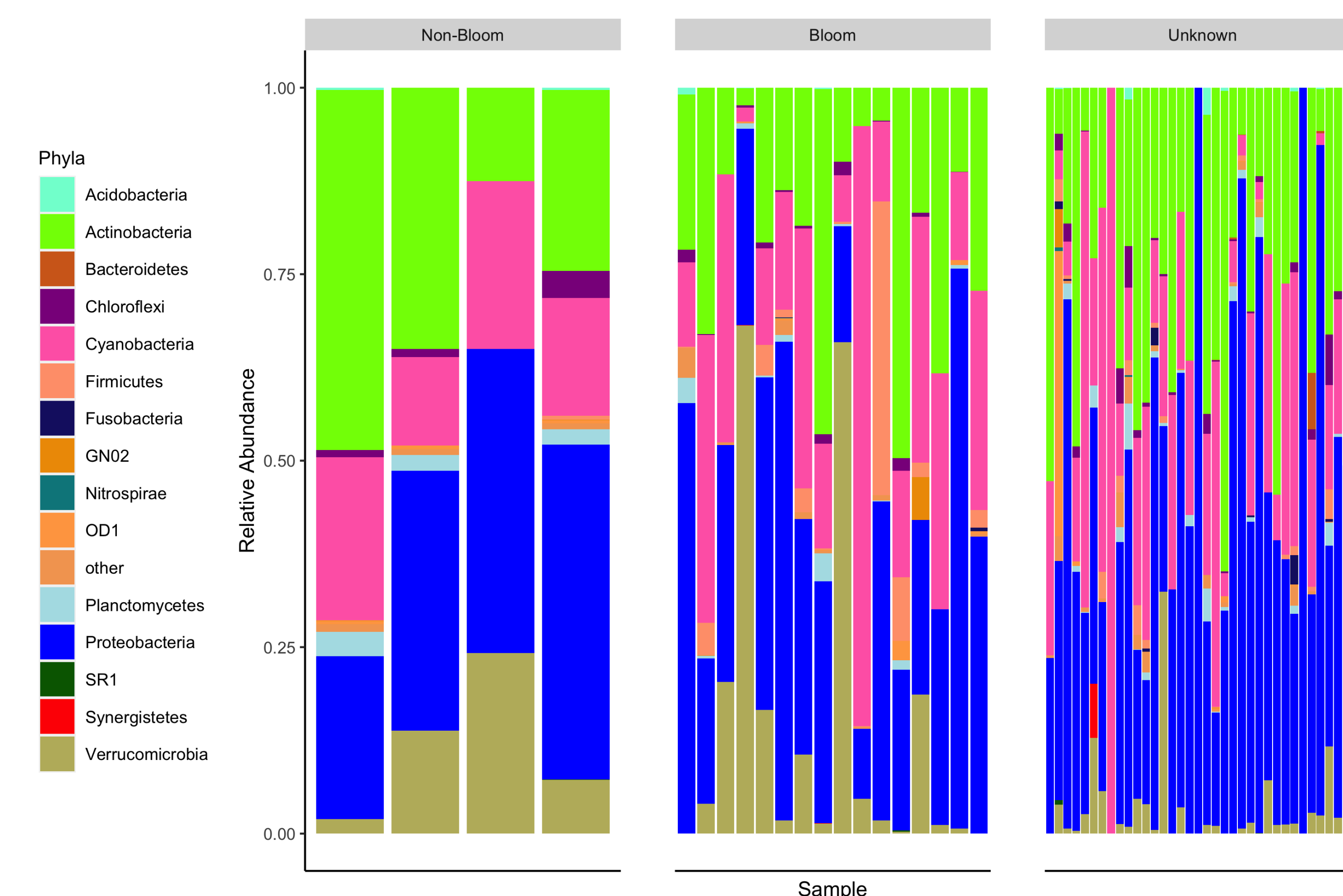


Figure 3. Relative Abundance of 16s rRNA Microbial Community Phyla in Bloom, Non-Bloom, and Unknown Sites

Future Work

- ⇒ Uncovering gene level differences in the microcystin biosynthesis gene operon of toxic and non-toxic genera of cyanobacteria.
- ⇒ Assessing the differences in composition of algal blooms based on factors such as location, time of year, and temperature.
- ⇒ Identifying not only taxonomic differences, but also functional differences between bloom and non-bloom environments.
- ⇒ Utilizing metagenomic approaches to characterize and monitor harmful algal blooms in combination with water quality and environmental data to better understand and predict the toxicity of blooms.