

Title: Stoichiometric Plasticity of Heterotrophic Bacteria in the Laurentian Great Lakes: The Impact of Nutrient Concentration on Microbial Community Adaptation

Background: The Laurentian Great Lakes represent the largest freshwater ecosystem on Earth and support around 34 million people and their economies, while providing crucial habitat for biological communities [1]. Influxes of nutrients and carbon (C) have impacted the lakes as a result of human population growth and land use change. The most affected lakes are Erie and Michigan, which receive the highest nitrogen (N) (61.5 and 62.9 kt/y, respectively) and phosphorus (P) (2.4 and 2.3 kt/y, respectively) inputs as particulate organic matter (POM), with the majority being runoff from manure and chemical fertilizers used for agriculture[2]. Changes in N and P cycling may lead to changes in C cycling, as C, N, and P cycles are often coupled [3], [4]. The impacts of rising nutrient inputs on biological communities and nutrient and carbon cycling are poorly understood. Therefore, we must investigate how microbially mediated ecological and biogeochemical processes concerning C, N, and P cycling affect the Great Lakes, and how changes induced by increased nutrient inputs will continue to impact the water quality of the Great Lakes. Microbial communities are of particular interest since they are heavily involved in nutrient cycling, carrying out processes such as nitrogen fixation, nitrification, and denitrification, as well as phosphate solubilization and organic matter decomposition, thereby making them essential controllers of nutrient availability and cycling. The composition of microbial assemblages can fluctuate [5], [6], along with their metabolisms [7], morphology [8], and stoichiometry [9], [10], as responses to environmental variables such as dissolved organic matter (DOM) and nutrients. Additionally, changes in stoichiometric ratios (C:N:P) can have impacts on the quality of food that lower trophic levels (bacteria and phytoplankton) provide for higher trophic levels (i.e., zooplankton and fish). It has been established that bacterial stoichiometry, metabolic capacity, and morphology change in response to particulate and dissolved C, N, and P in the environment, but these studies are often done *in vitro*, making it difficult to account for natural conditions. Here, I propose an *in situ* reciprocal transplant experiment where microbial communities from Lake Huron and Lake Superior will be collected and exposed to the nutrient conditions of each lake, to uncover the innate ability of microbial communities to adapt to changing nutrient conditions. In doing so, the proposed research will help inform management agencies on the present state of water quality in Lakes Huron and Superior while providing information on how increased nutrients into the Great Lakes may affect biological communities and important biogeochemical cycling.

Goals and Hypothesis: The goal of the proposed research is to investigate microbial community adaptation to different nutrient and DOM concentrations. **Objective 1:** Characterize the response of microbial stoichiometry to changes in POM and DOM. **Hypothesis 1 (H1):** Microbial communities from oligotrophic systems will be less flexible in their stoichiometry when compared to communities from eutrophic systems. **Objective 2:** Evaluate microbial community adaptation to altered nutrient concentrations and environmental conditions **Hypothesis 2 (H2):** Communities from oligotrophic systems will have lower functional redundancy compared to those from eutrophic systems, marked by the presence of more rare taxa and ability to metabolize a variety of carbon sources. **Hypothesis 3 (H3):** Communities taken from eutrophic environments will be less active in oligotrophic environments due to a limited range of OM substrates available.

Experimental Design: To investigate **H1**, water from Lakes Superior and Huron will be collected and used for an *in situ* reciprocal transplant experiment using dialysis bags (14,000 kDa MWCO). Water from one lake will be filtered using a 10 µm filter to remove grazers and placed

into dialysis bags, then incubated in the other lake for 14 days. Water samples taken from the bags will be analyzed for particulate C, N, and P of the bacterial communities and the seston. To measure DOM and dissolved nutrients, 0.45 µm filtered water samples will be analyzed for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), nitrogen species (nitrate, nitrite, and ammonium), and soluble reactive phosphorus (SRP). The quality of dissolved organic matter will be characterized by fluorescence excitation-emission matrix spectroscopy. To assess **H2**, morphological traits will be determined via flow cytometry, changes in community assemblage will be characterized using 16S rRNA gene sequencing, and functional capacity will be measured by carbon substrate utilization using BIOLOG Ecoplates. To explore **H3**, translationally active microbes will be quantified using biorthogonal amino acid tagging (BONCAT), which will provide information on microbial activity and whether the communities become more active or dormant in response to varying nutrient statuses. CTD and light profiles for each lake will also be collected at the beginning and end of each incubation for each host lake. Community adaptation in **H1** will be measured via plasticity in stoichiometry and morphology, while **H2** and **H3** adaptation will be reflected by microbial community assemblages and functional redundancy. Bray-Curtis dissimilarity will be used to assess similarity across native lake communities and incubated communities. The ratio of C, N, and P in the seston and transplanted communities will be calculated and compared to their lake of origin and the other transplanted community. Cell abundances and cell DNA content will be determined via flow cytometry, and transplanted communities compared to host communities. Finally, the data generated from this experiment will be used to make ordinations fitted with environmental variables to assess which variables are responsible for microbial community adaptation.

Timeline: Sampling will be conducted in May of 2026. Data analysis will be done in the fall of 2026. In Spring 2027, a manuscript will be drafted for publication. The results of this study will be included as a chapter in my dissertation, and I will tentatively defend it in spring 2029.

Products: The outlined experiments and their findings will be incorporated into my dissertation as a chapter and presented at the 2028 IAGLR meeting. Finally, a manuscript will be prepared and submitted to the *Journal of Great Lakes Research*.

Relation to MISG strategic plan: The proposed research is directly related to the 2024-2027 Michigan Sea Grant Strategic plan **Goal 1, Desired Outcome 1.2**, “Educators, students, and lifelong learners have current information and innovative tools that meet or exceed relevant standards and practices”. **Goal 3, Desired Outcome 3.2**, “Evidence-based science, traditional and local, and innovative solutions inform and improve management and conservation of coastal habitats”. **Goal 7, Desired Outcome 7.1**, “Scientific understanding, including traditional and local knowledge, provides foundational information, and all community members understand the impacts of changing conditions and coastal hazards and can prepare, respond, and adapt”.

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