Article Type

Letter

Hydrodynamic Circulation, Ecological Drift, and Homogeneous Selection Shape Microbial Communities and Biogeochemical Cycling in Lake Ontario

Running Head: Microbial Ecology of Lake Ontario

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Author Contribution Statement: Augustus Pendleton was responsible for conceptualization, methodology, software, formal analysis, investigation, visualization, data curation, and writing. Marian L. Schmidt contributed to the conceptualization, methodology, investigation, visualization, resources, data curation, writing, supervision, project administration, and funding acquisition.

Scientific Significance Statement

Microbial communities are essential for maintaining healthy aquatic ecosystems, forming the foundation of the food web, and regulating water quality through nutrient cycling. Large lakes, such as the Laurentian Great Lakes, display considerable intra-lake variability, characterized by nearshore-offshore gradients, point sources like cities and rivers, and spatial heterogeneity driven by seasonal hydrodynamic circulation patterns. However, there is still a gap in understanding how spatial variability impacts microbial abundance and community composition, along with implications for microbial metabolism and nutrient cycling. Using metabarcoding, flow cytometry, and water quality analyses, we mapped microbial biodiversity in Lake Ontario, achieving novel spatial breadth and resolution. Our findings emphasize the critical role that stratification and circulation play in shaping microbial communities and their ability to cycle nutrients.

Data Availability Statement

All raw and processed data for this project are publicly available. The main GitHub repository for this project will be shared upon acceptance, which includes all processed data (Amplicon Sequence Variants (ASVs), CTD casts, water quality data, all other metadata, etc.), the code for all figures, tables, and summary statistics, including the generation of ASVs using the DADA2 workflow, and a reproducible *renv* environment. All the software versions and citations used for data processing and statistics are described in Table S2. The raw compressed 16S rRNA gene sequencing fastq files are available in the NCBI Sequence Read Archive under the BioProject accession numbers PRJNA1212049. All flow cytometry data are available in the FlowRepository database with the ID number FR-FCM-Z8SJ (Spidlen et al. 2012).

Abstract

Microbial communities are vital to the ecology of the Laurentian Great Lakes, yet the influence of point sources, community assembly processes, and hydrodynamic circulation on their abundance and structure remains poorly understood. To address this, we analyzed 16S rDNA gene sequencing, flow cytometry, and water quality data from Lake Ontario during May and September of 2023. Communities were grouped into three depth-month categories: Shallow May, Shallow September, and Deep. Deep communities were dominated by drift, with some dispersal limitation, while shallow communities were shaped by homogenizing selection, especially in September, with contributions from drift. Actinobacteria were associated with homogenizing selection, Alphaproteobacteria with drift, and Bacteroidia in May and Chloroflexi in September with dispersal limitation. Upwellings altered microbial abundance and composition, modifying nitrogen cycling gene abundance. These findings provide insights into the ecological implications for Great Lakes health and conservation, emphasizing how upwelling events create unique microbial communities within the lake.

Keywords

Great Lakes - Metacommunity - Microbial Communities - Circulation - Biodiversity - Bacterioplankton

Introduction

Climate change is reshaping the ecology of the Great Lakes. Decreased ice cover and rapidly warming waters are disrupting stratification, circulation, and mixing patterns (Ozersky et al. 2021; Woolway et al. 2021; Woolway and Merchant 2019; Hlevca et al. 2024; Fichot et al. 2019). These changes modify species distributions and their metabolic rates and growth, impacting biogeochemical cycling and ecological dynamics which cascade through the food web (Magnuson et al. 1997). Due to their fast growth and metabolic diversity, microbes are quick responders to environmental change and play a critical role in regulating nutrient cycles and water quality. The intensity and direction of a microbial community's response to a disturbance, such as climate change, is influenced by their community assembly mechanisms (Langenheder and Lindström 2019).

Microbial communities form through selection, dispersal, diversification, and drift (Hanson et al. 2012; Hubbell 2011; Leibold et al. 2004; Martiny et al. 2006; Mittelbach and McGill 2019). Selection (*i.e.* species sorting or environmental filtering) is deterministic, with environmental factors shaping communities predictably (Chase and Myers 2011). Selection can homogenizing, fostering similar communities in comparable environments, or heterogenizing, resulting in dissimilar communities in variable environments (Vellend 2010). Alternatively, stochastic (or neutral) processes involve the probabilistic dispersal of species as well as unpredictable changes driven by random birth and death (Vellend 2010; Zhou and Ning 2017). Dispersal can be constrained by physical barriers or facilitated by mixing, enhancing homogenization among communities—at its strongest, a phenomenon known as "mass effects" (Vellend 2010; Langenheder and Lindström 2019). While typically stochastic, dispersal can be deterministic for taxa with traits that enable long-distance dispersal like spore formation. Diversification, which introduces novel species diversity into communities, is often considered stochastic in microbial ecology because of microbes' high diversity and rapid mutation rates though can also be deterministic (Vellend 2010; Zhou and Ning 2017). Drift is purely stochastic, causing random fluctuations in species abundances that drive community change. These assembly processes often overlap, interact, and operate simultaneously, which can be effectively eval-

uated using high-throughput sequencing in microbial communities (Stegen et al. 2013; Ofiţeru et al. 2010; Dumbrell et al. 2010).

The balance between deterministic and stochastic processes in shaping freshwater microbial communities is an active area of research, particularly as climate change and human activities alter environmental conditions. Understanding these dynamics is crucial for predicting the future function of freshwater ecosystems, including the Great Lakes. Studies have examined how freshwater gradients, such as waterbody size, watershed characteristics, urbanization, and seasonal changes, influence community assembly (Li et al. 2017; Isabwe et al. 2022; Ruiz-González, Niño-García, and del Giorgio 2015a; Liao et al. 2017; Chen et al. 2019; Roguet et al. 2015; Gu et al. 2021). Early research, focusing on abundant species, found limited evidence of dispersal limitation (Dolan 2005; Van der Gucht et al. 2007; Anthony C. Yannarell and Triplett 2004). However, advances in molecular techniques have highlighted the roles of dispersal limitation and drift, particularly for rarer species (Liao et al. 2017; Niño-García, Ruiz-González, and del Giorgio 2016a).

A developing model suggests that small waterbodies and headwaters, with shorter residence times and smaller population sizes, are more susceptible to stochastic processes like drift (Heino et al. 2015; Langenheder and Lindström 2019). Conversely, larger lakes, with longer residence times, often exhibit stronger selection, leading to more homogeneous communities (Heino et al. 2015; Niño-García, Ruiz-González, and del Giorgio 2016a). Yet, ecological drift can be a significant, even dominant, force in community assembly across freshwater systems, regardless of size (Roguet et al. 2015). Research has often focused on microbial assemblages *across* multiple lakes or within a watershed, primarily examining surface waters and treating individual lakes as homogeneous systems (Langenheder and Lindström 2019; Jones et al. 2012). This approach overlooks the considerable internal variation within lakes, which persists even in smaller lakes (~1 hectare) (Jones et al. 2012; Anthony C. Yannarell and Triplett 2004; Shade, Jones, and McMahon 2008). A key unanswered question remains: to what extent do such intra-lake variations influence microbial community assembly?

Lake Ontario, the easternmost of the Laurentian Great Lakes, offers a compelling study system for bacterial community assembly influenced by both stochastic and deterministic forces. Its annual stratification is shaped by well-characterized currents and circulation patterns, including upwelling events that transport nutrients from deeper waters to the surface, (Hui et al. 2022; Haffner et al. 1984; Hlevca et al. 2024), potentially creating distinct patches of microbial activity. Spanning nearly 20,000 km^2 (Woolway and Merchant 2018), Lake Ontario encompasses a diverse range of habitats, from relatively pristine shorelines to highly urbanized areas, exposing microbes to varied environmental pressures (Rozmarynowycz et al. 2019; Crevecoeur et al. 2023; Robertson et al. 2019). Pronounced nutrient and productivity gradients also exist, driven by nutrient transport from the Niagara River, nearshore-offshore gradients, urban point-sources, and the formation of thermal bars in spring (Makarewicz, Lewis, and Boyer 2012; Hui 2021; Neilson and Stevens 1987). The introduction of Dreissenid mussels has reshaped the lake's biogeochemistry, creating a nearshore nutrient shunt that intensifies offshore oligotrophy (Hecky et al. 2004; Dove and Chapra 2015). These features make Lake Ontario, and the Great Lakes more broadly, invaluable for understanding microbial community assembly in large, complex freshwater ecosystems.

In this study, we investigated microbial community assembly in Lake Ontario using samples collected in May and September 2023. Combining 16S rRNA gene sequencing and flow cytometry, we quantified the absolute abundances of prokaryotic species across diverse locations, depths, and environmental conditions. Our analysis revealed that homogenizing selection and drift were the dominant forces shaping microbial communities. Evidence of dispersal limitation was observed in the hypolimnion, particularly in September, where reduced mixing created distinct deep-water microbial communities. Seasonal circulation dynamics, including southern shore downwellings in May and a stronger southern shore upwelling event in September, significantly disrupted microbial community composition. These findings offer insights into the processes driving intra-lake bacterial community assembly and highlight the critical role of circulation in shaping microbial distributions in large lakes like Lake Ontario.

Methods

Sample collection

Samples were collected from multiple stations throughout Lake Ontario aboard the *R/V Lake Guardian* in May and September of 2023 as part of the US Environmental Protection Agency's (EPA) Lower Food Web Cooperative Science and Monitoring Initiative (CSMI) survey (Fig. S1; Table S1). The stations were arranged in 5 north-south transects across the lake, which were sampled in an east-west fashion over May 15th-19th and September 23rd-27th. Water samples were collected using a rosette with twelve 8 L Niskin bottles while depth profiles were taken with a CTD meter equipped with fluorometer, dissolved oxygen, spherical photosynthetic active radiation sensor, and turbidity sensors. "Surface" samples were from 5 m below the surface. "Mid" samples were collected at stations <30 m, either at the fluorescence maximum or thermocline in May or at the station's average depth in September. "Bottom" samples were taken 2 m above the total depth of a station. Water samples were taken in duplicate from separate niskins and pre-filtered consecutively through sterilized 200 μm and 20 μm Nitex mesh (Wildco) into sterile 10L bottles (Nalgene). Bottles were rinsed twice with sample water before filling with the rest of the water from the Niskin.

Microbial samples were filtered using MasterFlex Peristaltic Pumps at maximum pressure in size 24 Masterflex L/S tubing (VWR) onto a polyethersulfone 0.22 μm filter (MilliporeSigma). Samples were filtered for a maximum of 25 minutes, with flow through volumes ranging from 1500 mL to 8760 mL. Filters were cut into quarters, flash frozen in liquid N_2, and stored at -80C upon arrival back at the lab.

Environmental data

Water chemistry and chlorophyll a data were generated by the U.S. EPA's Great Lakes National Program Office according to their standard protocols (Agency 2003). See the Supporting Information for details on environmental data, including temperature monitors and meteorological records.

Quantifying cells with flow cytometry

Flow cytometry samples were prepared in the field by adding 1 μL (in May) or 5 μL (in September) of 25% glutaraldehyde to 1 mL of sample water. Samples were then shaken, incubated at room temperature for 10 minutes in the dark, and flash frozen in liquid N_2. Fixed samples were stained with SYBR I green dye and cell abundance was measured in DNA-positive cells with a BD Accuri C6 Flow Cytometer in triplicate. Gates identified fluorescently-labeled cells compared to unstained controls, allowing for background fluorescence from pigmented environmental cells (Fig. S2). Flow data was analyzed using the R package flowCore and ggcyto (Ellis et al. 2024; Van et al. 2018). Additional details are provided in the supporting information.

DNA extraction & Illumina sequencing

All DNA extractions were carried out using the Qiagen DNeasy PowerWater kit, mostly following the manufactuer recommendations (more details in Supporting Information). Half a filter was used for May samples and a quarter filter was used for September samples, translating to a minimum of 540mL extracted, with a median of 1320mL. An extraction negative was produced for each kit using a blank filter.

The V4 region of the 16S rRNA gene was amplified using the Earth Microbiome Project protocol (Apprill et al. 2015; Caporaso et al. 2011; Parada, Needham, and Fuhrman 2016). Polymerase chain reaction (PCR) was performed, with blanks, in triplicate 25 μL reactions using KAPA HiFi 2X MasterMix (Roche), 0.2 μM 515F (5'-GTGYCAGCMGCCGCGGTAA) and 806R (5'-GGACTACNVGGGTWTCTAAT) primers with Illumina Nextera adapter overhangs, and 5 ng of sample DNA. A Zymo-BIOMICS Microbial Community DNA Standard (Zymo) was included to assess the sequencing error rate. Samples and indexing blanks were sequenced using a 2 x 250 bp paired-end strategy on an Illumina MiSeq at the Cornell Biotechnology Resources Center, generating 15.72 million reads and a median sequencing depth of 105,500 reads (excluding blanks).

Microbial bioinformatic processing

Raw Illumina sequences were processed into amplicon sequence variants (ASVs) using the standard workflow in dada2 package in R (Callahan et al. 2016). The phyloseq package was used to organize the ASV count table, metadata, and later taxonomic table and phylogeny (McMurdie et al. 2022). Taxonomy was first assigned using the TaxAss freshwater database (Rohwer et al. 2018) and then with the Silva 16S rRNA database (Quast et al. 2013). Mitochondrial, chloroplast, and ASVs with a higher relative abundance in blanks were removed. In the mock community, three spurious ASVs were detected, representing a sequencing error rate of 0.03%. The resulting minimum number of reads in a sample was 7,931 reads, with a maximum of 73,652 reads, and a total of 7,280 unique ASVs.

A phylogenetic tree was constructed using MAFFT for alignment and FastTree under a generalized time-reversible model (Katoh and Standley 2013; Price, Dehal, and Arkin 2010). The tree was rooted at the most-recent-common-ancestor (MRCA) of the Archaea using the ape package (Paradis and Schliep 2019). Several polyphyletic, anomalous tips had exceptionally long branch lengths and no taxonomic assignment and were removed by filtering for tips with node heights greater than 2 as calculated using the `phytools::nodeheight` function (Revell 2024).

Ecological Statistics

Between-sample (beta) diversity was calculated using the abundance-unweighted and -weighted UniFrac dissimilarity metrics (Lozupone and Knight 2008) using the `vegan::distance()` command (Oksanen et al. 2022). The relative abundance of each ASV in a given sample was normalized by the cell concentration of that sample, providing the absolute abundance of each ASV. Ordinations were constructed using principal coordinates analysis implemented in phyloseq (McMurdie and Holmes 2013). Replicates were highly similar to each other (Fig. S3) and were merged by summing reads, resulting in a minimum of 13986 reads and a median of 82765 reads. Depth-month groups were defined with the weighted UniFrac dissimilarity using a UPGMA hierarchical

clustering method, cutting the tree at 3 groups (Fig. S4A). Variance partitioning and post-hoc tests were performed with Vegan functions `varpart`, `dbrda`, and `anova.cca` (Oksanen et al. 2022). Permutational multivariate analysis of variance (PERMANOVA) and multivariate homogeneity of groups dispersions tests were run with `adonis2` and `betadisper` from the `vegan` package (Oksanen et al. 2022).

Within-sample (alpha) diversity was estimated using the effective Hill numbers—richness, exp(Shannon), and the Inverse Simpson Index—with the iNEXT package, at orders `q = c(0,1,2), datatype = "abundance"` (Chao et al. 2014; Roswell, Dushoff, and Winfree 2021). All rarefaction curves were asymptotic, confirming sufficient sequencing depth for representative sample richness (Fig. S5).

Differential abundance of microbial ASVs between depth-month groups was calculated at the Class level using the pairwise setting in the ANCOM-BC2 package (H. Lin, Eggesbo, and Peddada 2022). Significant differences had an FDR-corrected p-value less than 0.05 and passed the sensitivity test across multiple pseudocount values; other parameters were kept at default settings.

Microbial community assembly processes were quantified with iCAMP (Ning et al. 2020). To account for variability within sub-populations of a community, iCAMP divides the community into smaller, monophyletic bins containing several ASVs. We used a maximum bin size of 24 ASVs, a maximum bin distance of 0.4, and a confidence threshold of 0.975 compared to a randomized null distribution with 1000 iterations (Ning et al. 2020). Briefly, within each bin, the relative importance of each assembly processes is evaluated. Selective processes were quantified using the β Net Relatedness Index (βNRI). βNRI values $<$ -1.96 indicates homogeneous selection, whereas values $>$ +1.96 indicates heterogeneous selection. βNRI values between -1.96 and +1.96 suggest that selection is unimportant, rather dispersal processes were assessed using a modified Raup-Crick metric (RC_{bray}), which incorporates taxon abundances (Stegen et al. 2013; Ning et al. 2020). An RC_{bray} value $>$ +0.95 indicates dispersal limitation, $<$ -0.95 reflects homogeneous dispersal, and values between -0.95 and +0.95 suggest stochastic processes or ecological drift.

The relative importance of each assembly process in community turnover was estimated by summing the bins dominated by that process, weighted by the overall abundance of those bins. Details on iCAMP are in the Supporting Information.

Microbial functional traits were inferred using PICRUSt2 (Douglas et al. 2020), using the absolute abundance of each ASV and max Nearest Sequenced Taxon Index (NSTI) values of both 0.2 (phylogenetically broad, used 97% of ASVs) and 0.03 (phylogenetically stringent, used 13%). Overall correlations between the abundance of inferred pathways between both settings was similar, with a median correlation of 0.92 across 409 pathways. As such, we kept the more inclusive results (NSTI 0.2). We filtered the inferred abundance of nitrogen cycling genes by querying genes known to be essential and exclusive for a given nitrogen transformation, including nitrogen fixation (*nifH*), ammonia oxidation (*pmoA/amoA*), nitrite oxidation (*narG, narZ, nxrA* cluster), and urea hydrolysis (*ureC*). See Supporting Information for more details.

Statistical testing, including Two-Sample Wilcoxon tests following Kruskal-Wallis tests; correlation tests; and linear models were done in R, using base functions and the ggpubr package (Kassambara 2023). If multiple comparisons occurred within a given plot panel, all p-values were corrected with a Bonferroni Correction.

Spatial Analysis

The spatial distribution of physicochemical parameters, community dissimilarity, and nitrogen gene abundance were analyzed using the packages sf, terra, tmap, tmaptools and gstat in R (E. Pebesma 2018; Hijmans 2024; Tennekes 2018, 2021; E. J. Pebesma 2004), and interpolation was performed using either inverse distance weighted interpolation in R or multi-level B-spline interpolation with GRASS implemented in QGIS (E. Pebesma 2018; Hijmans 2024; Tennekes 2018; GRASS Development Team 2020; QGIS Development Team 2021). While spline steps and Tikhonov smoothing varied, these parameters were kept consistent within each figure. Data files for Lake Ontario's outline and bathymetry were downloaded from United States Geological Society and National Oceanic and Atmospheric Administration, respectively (USGS, n.d.; National Geophysi-

cal Data Center and NOAA, n.d.).

Results

Upwellings are common in Lake Ontario

We detected upwelling events along the northern shore in May (stations 23 & 43) and along the southern shore in September (station 38) (Fig. 1A, 1B, & Fig. S6). In May, the warm southern shore likely composed a combination of downwelling and relatively warmer water flowing in from the Niagara River (Fig. S7A-C). In September, strong easterly winds in the days leading up to sampling likely explain the strong southern upwelling at station 38, which is reflected in both temperature (Figure 1B, Fig. S7D) and nutrient data. We estimate this upwelling formed near the Niagara outlet on September 23rd and propagated as a Kelvin wave along the Southern shore, concluding at the eastern terminus by October 9th (>2 weeks later), at which point winds shifted back from the west, causing upwellings along the northern shore (Fig. S7D-E).

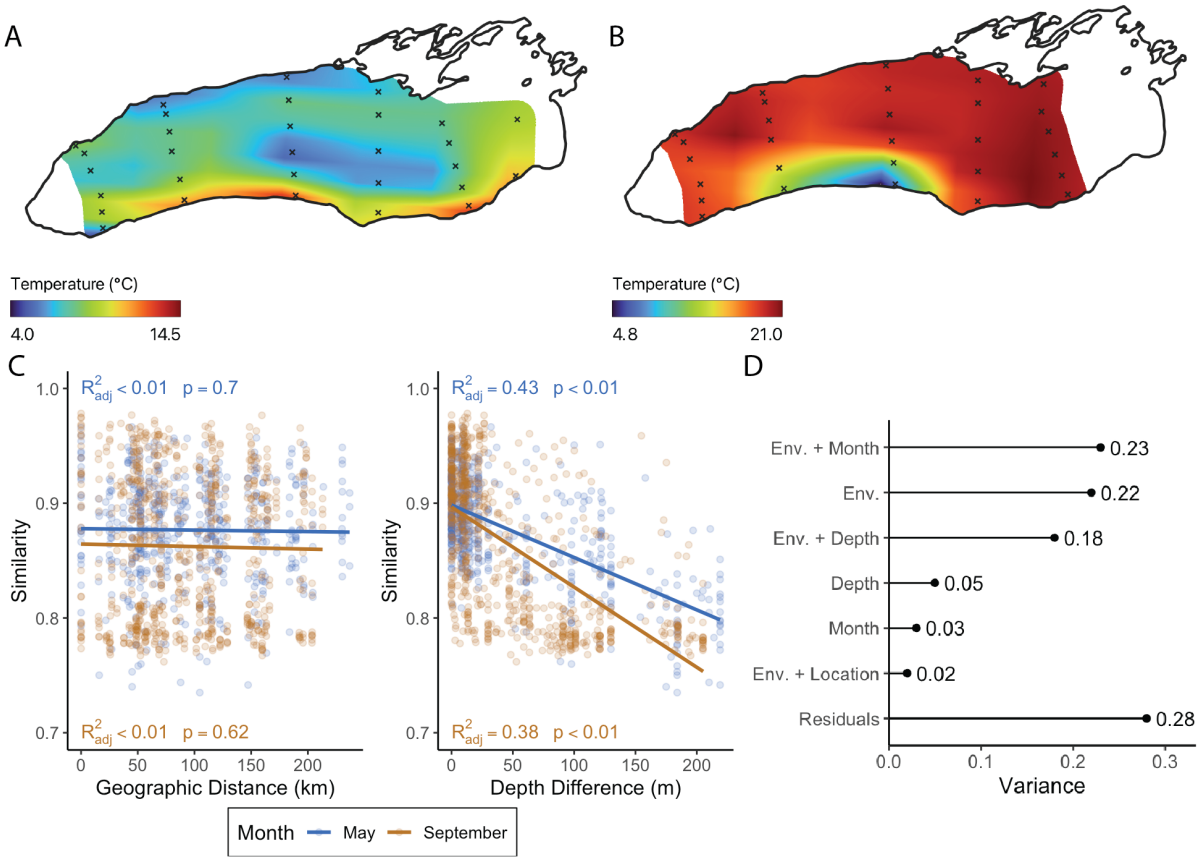

Figure 1. Spatial and depth variation in temperature structures Lake Ontario's microbial community. Shallow (5 m) temperatures in Lake Ontario interpolated spatially across the lake in (A) May and (B) September. Interpolation was performed using multilevel B-splines in QGIS/GRASS with 40 km east-west steps, 10km north-south steps, and a Tykhonov regularization of 0.01. Color scales vary between months. (C) Microbial community distance- and depth-decay relationships by month using similarity defined as 1 - weighted UniFrac dissimilarity. (D) Variance partitioning of the weighted UniFrac dissimilarity. Environmental variables (Env.) corresponded to scaled physical and chemical from each sample. Only variables which were significant (all $p < 0.001$) when tested using an ANOVA-like permutation test for Constrained Correspondence Analysis post-dbRDA were included in the plot.

Lake Ontario's microbial communities form three distinct groups defined by depth and season

Lake Ontario bacterial communities were clustered into three distinct based on the abundance-weighted Unifrac dissimilarity (Fig. 2A & S4). These groups closely to sample depth (typically >30 m for deep samples, depending on thermocline depth) and month of collection, and are thus referred to "depth-month groups." This pattern was consistent across both hierarchical clustering (Fig. 2A & S4A) and principal coordinates analysis (Fig. S4C). Deep samples from both months grouped together, while shallow samples from May and September formed distinct clusters ($p < 0.001$, $R^2 = 0.63$, PERMANOVA). Therefore, we categorized bacterial communities into three groups for analysis: Shallow May, Shallow September, and Deep.

Highest Microbial Abundance in Shallow September, near the Welland Canal, and unexpectedly in the pelagic zone

Microbial cell abundances were strongly correlated with temperature (Fig. S9A; Spearman's R =0.92, $p < 2.2e\text{-}16$). Cell abundances were highest in Shallow September, followed by Shallow May, and lowest in Deep samples (Fig. S9B). Unexpectedly, deep September samples had fewer cells than Deep May samples ($p = 0.031$, Two-Sample Wilcoxon). Spatial patterns of cell abundance varied across the lake surface in both May and September (Fig. S9C). In May, cold, upwelling areas along the northern shore had lower cell counts while warmer offshore areas and the southern shore downwelling and Niagara-river impacted regions exhibited higher cell counts. In September, the upwelling zone at station 38 had the lowest cells abundances, whereas station 17, influenced by

Welland Canal water, had the highest cell abundances.

Environmental conditions, not geographic location, shape bacterial community composition

Bacterial communities assembly was examined across spatial and environmental gradients during May and September. Community similarity (1 - Weighted UniFrac Dissimilarity) was not correlated with geographic distance but exhibited a strong relationship with depth in both months, particularly when the thermocline intensified in September (Fig. 1C). Depth was a stronger predictor of bacterial community composition than geographic location.

Variance partitioning was used to quantify the influence of environmental factors on community composition (Fig. 1D). Depth, month, and other environmental variables explained 73% of the variance in weighted UniFrac dissimilarity (Fig. 1D), as supported by a PCA of the environmental variables (Fig. S4B). Geographic location, even when combined with environmental variables, accounted for only 2% of the variance. Lake Ontario's bacterial communities are primarily shaped by environmental factors, especially depth.

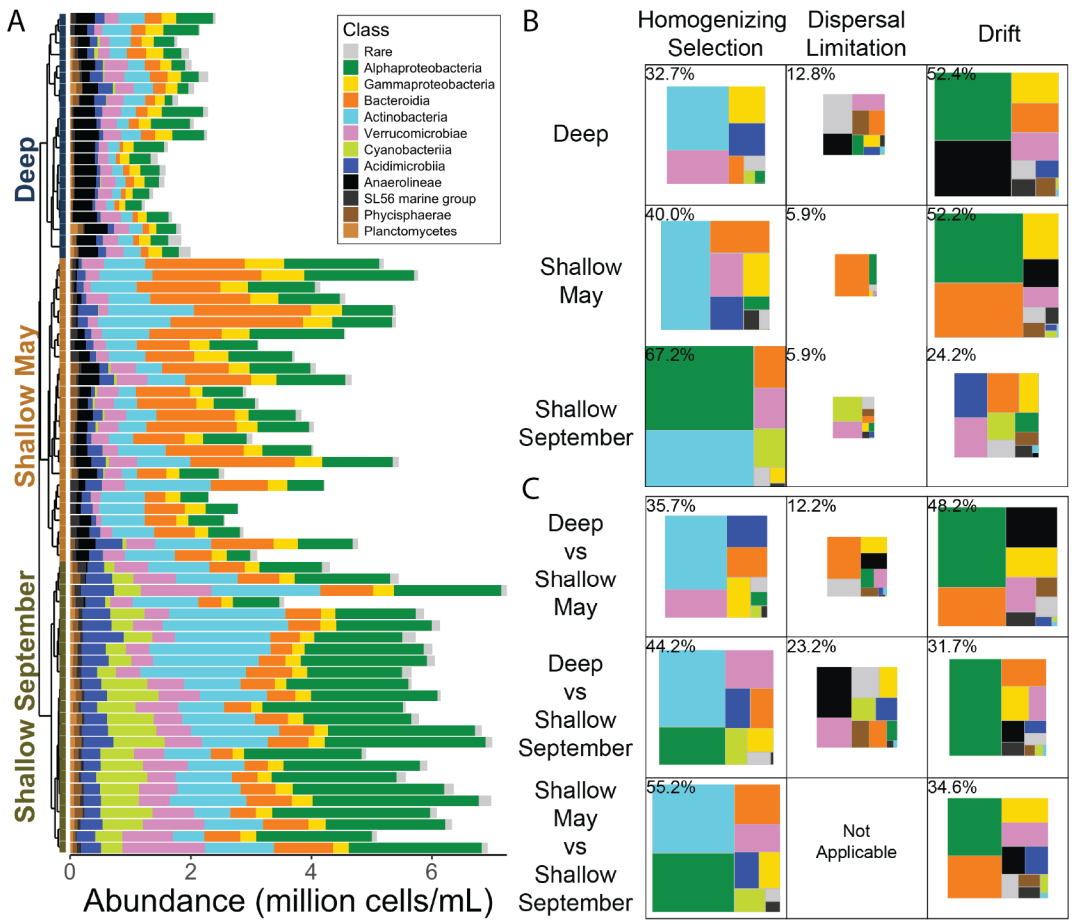

Figure 2. Homogenizing selection and drift drive Lake Ontario's microbial community assembly and composition. (A) Hierarchical clustering of the weighted UniFrac dissimilarity (same as Figure S4A), defining three depth-month groups. Each bar represents the total absolute abundance of ASVs in a sample, colored at the Class-level.(B) Relative contribution of different taxonomic groups to microbial community assembly processes, as determined by iCAMP. The area of each box represents the relative contribution of that process (columns) to turnovers between samples (B) *within* a given depth-month group, or (C) *between* two depth-month groups. The percentage contribution of a given process to community turnover is in the top left corner, and boxes are scaled relative to the most influential process (*i.e.* homogenizing selection in shallow September at 62.7%), and the area represented by each Class is scaled relative to that Class's contribution to that process.

Specific taxa are tied to distinct community assembly processes

Microbial communities in Lake Ontario shared a core set of cosmopolitan taxa, but certain Classes were closely tied to specific depth-month groups. Alphaproteobacteria (predominantly LD12, a close relative of the marine SAR11 lineage), Actinobacteria (including acI-B1, acI-C2, acI-A7, acI-B1, acI-A6, acSTL-A1, acI-A5, and acI-A3), Bacteroidia (such as unknown species and bacI-A1, bac-IIA, and bacIII-A), Gammaproteobacteria (LD28 and PnecB, formerly Betaproteobacteria), and Verrucomicrobiae (mostly LD19,

SH3-11, and unknown species) were ubiquitous across all samples (Figs. 2A & S10A-B).

Differential abundance analysis revealed distinct habitat preferences among taxa. Chloroflexi Anaerolineae, including an ASV sharing 99.6% identity with CL500-11 from Crater Lake (Urbach et al. 2001), were enriched in Deep and shallow May samples (Fig. S10B). Deep samples had elevated levels of nitrifiers, including the ammonia-oxidizing Archaea Nitrososphaeria and the nitrite-oxidizing Bacteria Nitrospiria. In contrast, Bacteroidia were more prevalent in shallow waters, with the largest populations in May. Acidimicrobiia and Cyanobacteria (mostly *Cyanobium PCC-6307*) were prominent in shallow September, along with two potentially harmful cyanobacterial genera (Fig. S11A-B). *Dolichospermum NIES41* was almost 1% of the community (>60,000 cells/mL) at station 17 near the Welland Canal (Fig. S11C), while *Microcystis PCC-7914* was abundant across multiple stations, peaking at ~100,000 cells/mL at station 43 near Cobourg, Ontario (Fig. S11D).

We linked differences in community composition to drift, dispersal, and selection with iCAMP. Drift dominated Deep samples (52%), with some influence from dispersal limitation (13%) (Fig. 2B). Dispersal limitation was more pronounced when comparing Deep to Shallow samples (12%), increasing to 23% in September during stronger stratification. In Shallow samples, homogenizing selection was greater, especially in September (67%) (Fig. 2B). Across depth-month groups, homogenizing selection explained 36%–55% of turnover, indicating consistent selection for a core microbial community throughout the lake (Fig. 2C).

Next, we examined how assembly processes corresponded to the Class-level taxonomic composition of each depth-month group (Fig. 2B&C). Homogenizing selection was primarily driven by the Actinobacteria, while drift was dominated by Alphaproteobacteria. Dispersal limitation between deep and shallow groups was primarily influenced by Bacteroidia in May and Anaerolineae in September.

Microbial abundance and community assembly processes are linked

The contribution of taxonomic groups to community assembly was closely tied to their abundance within samples(Fig. 3A), particularly for within-group comparisons. More abundant bins had lower abundance variation across samples (Spearman's ranked correlation, ρ = -0.61, p < 0.0001) and were primarily shaped by drift and homogenizing selection. In contrast, low-abundance bins with high variance were dominated by dispersal limitation, while intermediate-abundance bins were influenced by both drift and dispersal limitation.

Across Phyla, a bin's dominant assembly process was more influenced by abundance than by taxonomy, reflecting ecological niches rather than taxonomic conservation (Fig. 3B). However, bins with similar abundances occasionally had divergent processes. For example, the *bacV*, *bacI-A*, and *bacII-A* bins within the Bacteroidota were driven by drift, homogenizing selection, and a mix of dispersal limitation and drift, respectively, despite having similar abundances.

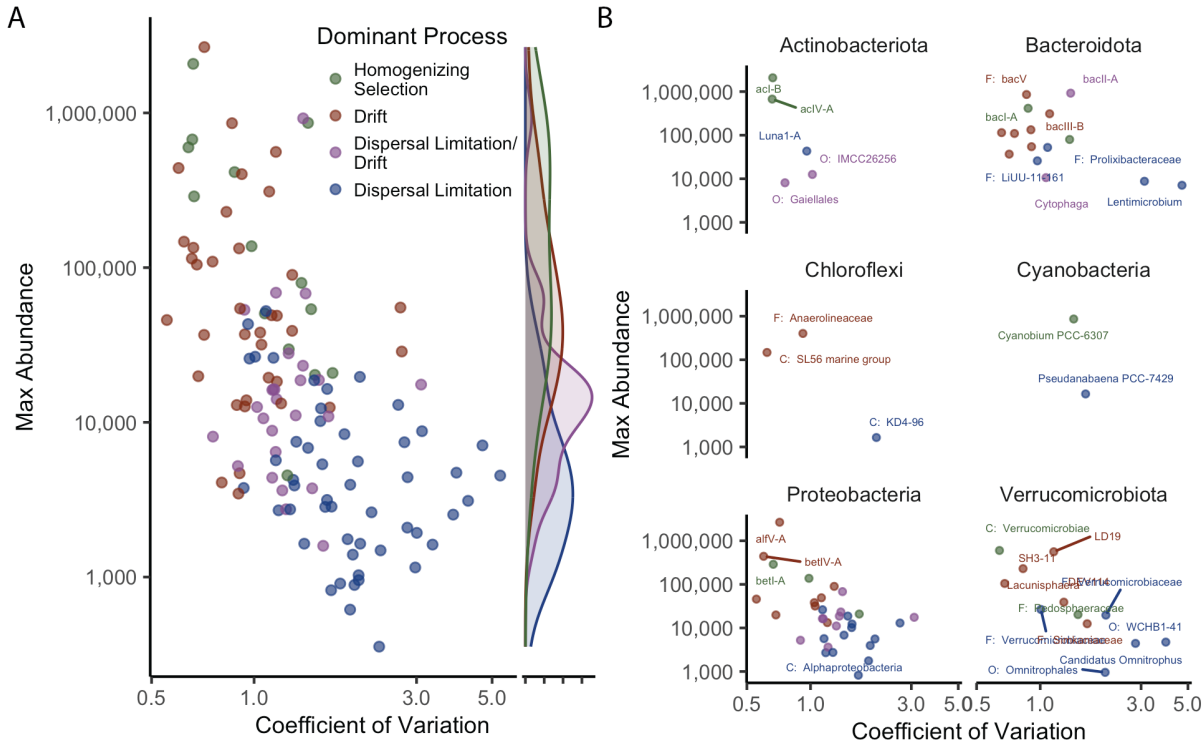

Figure 3. Microbial abundance, variance, and community assembly processes are interconnected. (A) The relative importance of community assembly processes within each phylogenetic bin, calculated by iCAMP. Absolute ASV counts were summed per bin per sample while the coefficient of variation was calculated

as the standard deviation of abundances across samples by the mean abundance of that bin across all samples. The density plot on the right shows the distribution of each process across the maximum phylogenetic bin abundances. Three bins dominated by heterogeneous selection or a combination of drift and homogenizing selection were excluded. (B) Data in (A), but faceted by the six most abundant phyla. Labels indicate species; if unavailable, Genus, Family, Order, or Class is specified using "G:", "F:", "O:", or "C:"

Upwelling creates unique microbial communities by enriching rare taxa

Our lakewide sampling revealed spatial relationships between environmental conditions, circulation patterns, and microbial community composition within Lake Ontario (Fig.4). In May, shallow communities were more dissimilar than in September (PERMDISP, p = 0.002). Distinct communities occurred near the Welland Canal and the northern station of Transect 4 (station 48; Fig. 4), where TN:TP ratios were anomalously high (Fig. S8C). In September, shallow communities were more similar, except along the southern shore, where upwelling created highly dissimilar communities (Fig. 4). The high dissimilarity was driven by an influx of hypolimnion–associated taxa (Fig. S4A&C), likely due to mass effects, and many rare taxa not observed elsewhere (Fig. S12A-C). Rare taxa were also abundant in May near the Welland Canal (Fig. S12A) but not along the northern shore upwelling (Fig. S12B).

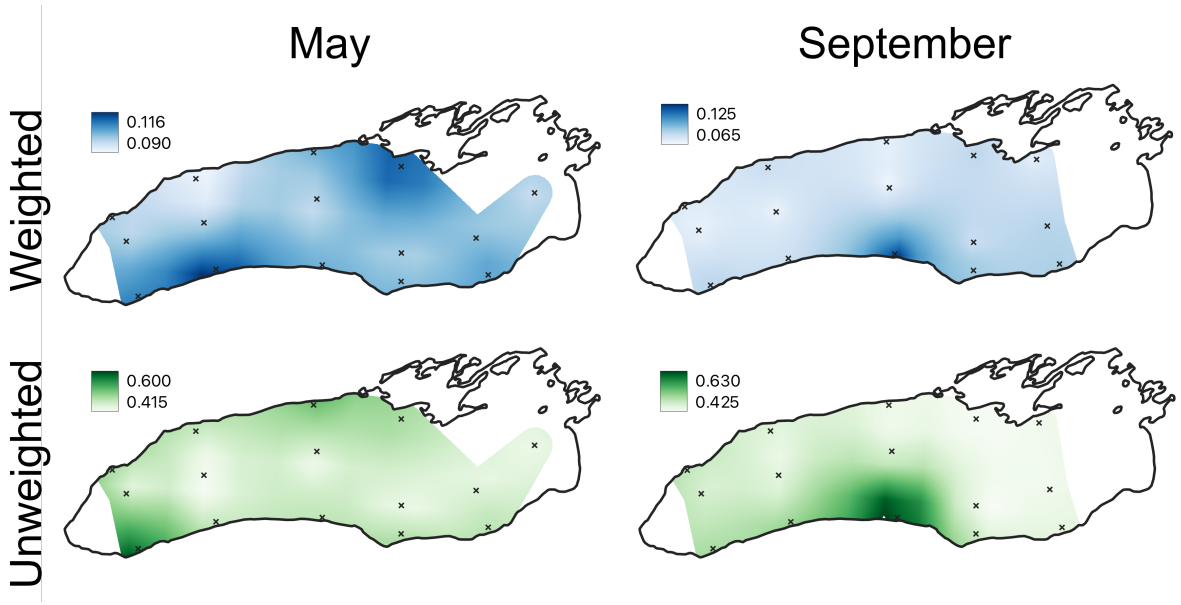

Figure 4. Circulation patterns strongly modify bacterial community composition. Average dissimilarity was calculated as the mean pairwise abundance-weighted (top) or -unweighted (bottom) UniFrac dissimilarity of each shallow (5 m) sample in May (left) or September (right) to all other shallow (5 m) samples from the same month. Interpolation was performed using multilevel B-splines in QGIS/GRASS with 20

km east-west steps, 10 km north-south steps, and a Tykhonov regularization of 0.05. Color scales are independent for each map.

Lakewide Circulation Shapes Microbial Nitrogen Cycling We used PICRUSt2 to infer gene abundances related to microbial nitrogen metabolism, including nitrogen fixation, ammonia oxidation, nitrite oxidation, and urea hydrolysis (Fig. 5 & Fig. S13). Urea hydrolysis was the most abundant annotated process, followed by nitrite oxidation, ammonia oxidation, and nitrogen fixation. Nitrogen fixation and urea hydrolysis were highest in shallow September, followed by shallow May, deep September, and finally deep May samples (Fig. S13). Urea hydrolysis had the highest per-capita abundance ($p = 0.00012$, $p = < 0.0001$, and $p = 0.0005$ compared to deep May, shallow May, and shallow September, respectively; Fig. 5A). Nitrite oxidation had the highest gene counts in shallow May followed by shallow September, and the deep samples (Fig. S13), though on a per-capita basis, only shallow September had significantly lower counts ($p < 0.0001$ for shallow September compared all other groups, $p > 0.05$ between deep samples and shallow May; Fig. 5A). Ammonia oxidation peaked in deep September ($p < 0.0001$ for all comparisons; Fig. S13), but was not significantly different between other depth-month groups.

Nitrogen gene abundances were poorly correlated with NO_x and NH_4 concentrations in surface waters (Fig. S14), indicating spatial structuring beyond environmental filtering (Fig. 5B). While responses varied by month, the Welland Canal and Niagara River plume had more nitrogen fixation (in September), nitrite oxidation (both May and September), and urea hydrolysis genes (in May) (Fig. 5B). Warmer waters were associated with higher nitrogen fixation and urea hydrolysis along the southern shore in May and increased urea hydrolysis offshore in September. Colder waters were associated with nitrification genes, aligning with higher ammonia oxidation gene counts observed in the deep waters of September and elevated nitrite oxidation genes in the shallow waters of May (Fig. S13). Nitrification hot spots shifted seasonally, concentrating genes in the southern upwelling and Welland Canal in September, while in May they were more prominent offshore and less abundant along the northern shore upwelling.

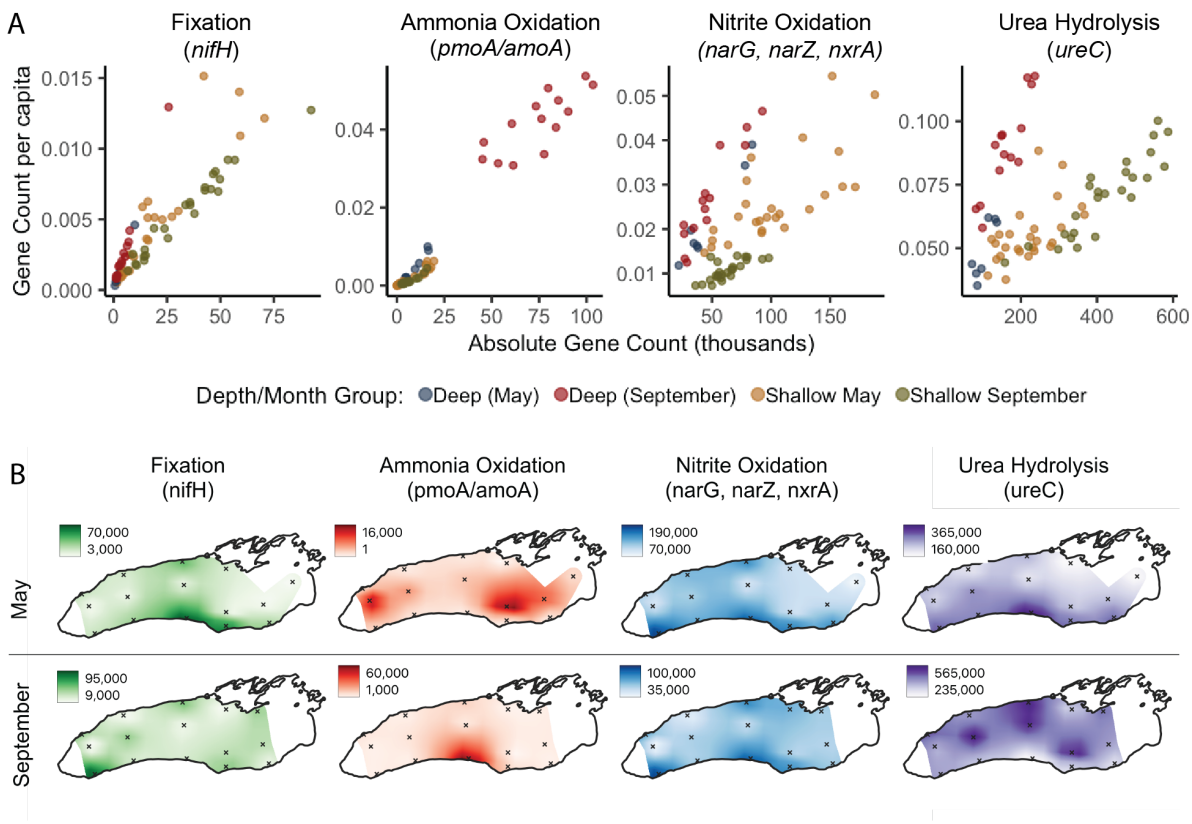

Figure 5. Circulation patterns strongly modify bacterial community assembly. (A) The abundance of genes representative of different nitrogen cycling processes was inferred using PICRUSt2 and normalized by the absolute abundance of the ASV from which it was predicted (Absolute Gene Count). Per-capita gene count was calculated by dividing the absolute gene count by the number of cells/mL in a given sample. (B) Interpolated absolute counts of genes involved in nitrogen cycling. Interpolation was performed using multilevel B-splines in QGIS/GRASS with 20km east-west steps, 10km north-south steps, and a Tykhonov regularization of 0.05. Note that color scales are independent between maps.

Discussion

Stochasticity, determinism, and circulation in Lake Ontario's surface waters

We explored how selection, dispersal, and drift shape microbial community assembly in Lake Ontario. We found that homogenizing selection and drift predominantly influenced surface waters, especially during warm late-summer conditions in September. In contrast, reduced mixing in the hypolimnion imposed dispersal limitation, creating stable yet distinct deep-water microbial communities. Seasonal circulation, including transient (but frequent) upwelling and downwelling events, disrupted microbial abundance and composition across the lake.

Lake Ontario's thermal structure in May and September revealed distinct circulation

patterns with differing effects on microbial communities. In May, surface conditions and microbial communities were more variable and similar to deep communities (Fig. S4A&C), likely reflecting ongoing niche partitioning after spring mixing. By September, surface communities were highly similar, driven by intense homogenizing selection within the epilimnion. These findings reflect the Pacific Ocean where homogeneous selection, and to a lesser extent dispersal limitation, were the strongest factors shaping free-living microbial communities, especially in surface waters (Milke et al. 2022). Our results further align with findings from oligotrophic lakes, where deterministic processes like selection dominate (Gu et al. 2021; Liao et al. 2017; Li et al. 2017; Aguilar and Sommaruga 2020), but contrast with more productive lakes, where stochasticity increases due to higher nutrient availability (Roguet et al. 2015; A. C. Yannarell et al. 2003; Drakare and Liess 2010).

We hypothesized that tributary inputs would create nearshore-offshore gradients in microbial composition, similar to patterns in Lake Michigan (Mueller-Spitz, Goetz, and McLellan 2009; Fujimoto et al. 2016) due to the overwhelming influx of terrestrial bacteria in small waterbodies (Wang et al. 2013; Ruiz-González, Niño-García, and del Giorgio 2015b; Niño-García, Ruiz-González, and del Giorgio 2016a; Isabwe et al. 2022; Chen et al. 2019). However, in Lake Ontario, tributary impacts were modest, with communities remaining consistent across the lake due to strong homogenizing selection (Fig. 4). An unweighted metric revealed tributary inputs from the Welland Canal and/or Niagara River in May (Fig. 4), likely increasing the diversity of rare microbes through dispersal (Fig. S12). Yet, these rare taxa were filtered away in the offshore, leading to a core community of abundant taxa dispersed throughout the lake. Tributary impacts may persist locally when combined with physical processes like downwelling or thermal bar formation (Fig. 1A, Fig. 5B), enriching nitrogen cycling (*see Supplemental Discussion: Nitrogen Cycling*).

In September, a upwelling event moved bottom-dwelling microbes and their metabolic processes to the surface. Upwelling events are more frequent along the northern shore, driven by prevailing south-westerly winds (Hlevca et al. 2024). While coastal marine

upwellings are known to fuel phytoplankton blooms and increase microbial richness and functional potential (Aldunate et al. 2018; Sun et al. 2020; Kerkhof et al. 1999), freshwater upwellings are less understood. In Lake Ontario, the September upwelling transported deep-associated bacteria (*e.g.*, Anaerolineae and Nitrospiria) and their functional genes (*e.g.* ammonia oxidation) to the surface, mixing with shallow-associated taxa like Cyanobacteriia (Fig. S15A), mirroring findings from seasonal upwellings in Lake Tanganyika (De Wever et al. 2005). The fate of these transported deep-water microbes remains unknown, warranting further study to track their survival and dispersal (*see Supplemental Discussion: The Upwelling Niche*).

Drift and dispersal limitation define the hypolimnion

Dispersal limitation, combined with drift, significantly contributed to community assembly in the hypolimnion. Stratification restricted migration between the epilimnion and hypolimnion (Fig. 2B), and limited dispersal within hypolimnion samples reflected weakened mixing in deep waters (Gbah, Rao, and Murthy 2001). Further, hypolimnion communities were more temporally consistent compared to the epilimnion (Fig. S4A&C). Previous studies have also highlighted reduced temporal variation in hypolimnion communities (Shade, Jones, and McMahon 2008; Okazaki and Nakano 2016) and greater compositional consistency spatially across Great Lakes basins (Paver, Newton, and Coleman 2020).

Drift's role in the hypolimnion is notable given the cold, oxygenated, and homogeneous conditions of oligotrophic hypolimnia, which typically favor deterministic processes [*see Supplemental Discussion: Selection in the Hypolimion*; Wang et al. (2013); Niño-García, Ruiz-González, and del Giorgio (2016a); Huber et al. (2020); Mueller and Lennon (n.d.)]. However, studies in the Pacific Ocean and elsewhere have shown drift's importance increases with depth (Milke et al. 2022), possibly due to reduced cell abundance (Evans, Martiny, and Allison 2017), consistent with the sparse microbial populations in Lake Ontario's hypolimnion (Fig. S9B). Dormancy in deep waters may further weaken selection (Lennon and Jones 2011; J.-H. Lin et al., n.d.).

Reduced mixing (*e.g.*, lower current velocities (Gbah, Rao, and Murthy 2001)) and low cell counts in the hypolimnion can drive stochastic changes, creating signals of dispersal limitation. However, we found no evidence of lakewide distance-decay patterns in either abundant or rare hypolimnion communities (Fig. S16E & S16F), suggesting that intra-lake variation reflects temporarily isolated parcels rather than consistent lakewide gradients (Jones et al. 2012).

Abundance and taxonomy influence community assembly processes

We found that community assembly mechanisms were closely linked to a given phylogenetic bin's abundance and prevalence. Drift and homogenizing selection was restricted to the most abundant taxa (Fig. 3A), aligning with studies highlighting the increased role of selection for abundant bacterioplankton (Li et al. 2017; Niño-García, Ruiz-González, and del Giorgio 2016b). In contrast, dispersal limitation dominated for rare taxa (*see Supplemental Discussion: Rarity in Lake Ontario*).

Taxonomy also influenced assembly processes: Actinobacteria were more associated with homogenizing selection, while Alphaproteobacteria were dominant in drift. This suggests greater niche partitioning among Actinobacteria compared to the stochastic, widespread distribution of Alphaproteobacteria, represented almost exclusively by LD12 (Fig. S10B). The higher diversity of abundant and prevalent Actinobacteria supports this distinction (Newton et al. 2011).

Community assembly processes work collaboratively, not individually, and rely on assumptions

While we focus on major drivers of community assembly, it is important to acknowledge that these processes are inter- and scale-dependent. Our broad spatial coverage may lack the resolution to detect fine-scale patterns, such as shoreline (<10m) communities (Fujimoto et al. 2016) and microniches (e.g., particle-associated vs. free-living, Yuan et al. (2021)). Additionally, with only two sampling points during stratified periods, we lacked the temporal resolution to capture changes during thermocline formation and dissolution

or to detect a deep chlorophyll layer. Whether similar assembly processes differentiate hypolimnion and epilimnion communities during winter inverse stratification also remains unclear (Ozersky et al. 2021; Pu et al. 2024). Greater temporal and spatial resolution is needed to further elucidate the annual dynamics of microbial community assembly. Finally, the inference of assembly processes using iCAMP is subject to methodological biases (*more discussion in Supplemental Discussion: Biases in iCAMP*).

An expanded picture of microbial diversity in Lake Ontario

Previous studies of Lake Ontario microbial diversity focused on a few offshore stations (Paver, Newton, and Coleman 2020; Winters et al. 2014; Mohiuddin et al. 2019; Gale et al. 2023) or highly impacted embayments (Palermo et al. 2023). Our findings confirm dominant taxa like the *acI* and *LD12* lineages throughout the lake, the oligotrophic heterotroph *CL500-11* in the hypolimnion, and non-toxic *Synechococcus* in summer surface waters. Notably, we observed an abundance of Bacteroidia (*Flavo-A*, *bacI*, and *bacII*) in spring and a previously unreported diversity of Acidomicrobiia (*acIV* lineages like *Iluma-A1*), expanding known microbial diversity of the lake. Though, these differences may reflect primer bias or the effects of a different pre-filtration strategy (*see Supplemental Discussion: Biases in Sampling and Sequencing*). Filamentous Bacteroidia may dominate in spring by averting grazing (Newton et al. 2011; J et al. 1996). Additionally, we detected the *acI-C2* lineage, characteristic of the lower Great Lakes (Paver, Newton, and Coleman 2020), which was significantly more abundant in shallow September (Fig. S17), suggesting niche selection to the relatively warmer lower lakes (Kim et al. 2021).

The future of microbial communities in Lake Ontario

Our findings reveal the critical role of stratification and circulation in structuring microbial communities in large lakes. As northern temperate lakes warm due to climate change, stratification is projected to lengthen and intensify (Woolway et al. 2021; Lehman 2002; Schwefel et al. 2019), modifying the size of the hypolimnion and its unique microbes, such as those specializing in ammonia oxidation. These shifts could significantly alter

microbial biogeochemical cycling, amplifying homogenizing selection in surface waters and dispersal limitation in bottom waters, intensifying differences between nutrient processing in the epilimnion and hypolimnion.

The impact of climate change on upwelling in the Great Lakes remains uncertain. While stronger stratification may suppress upwelling, increased wind activity could enhance it, as observed in marine systems (Iles et al. 2012). In Lake Ontario, upwelling-favorable winds have risen by 45% over 30 years (Jabbari et al. 2023), though future patterns remain unclear (Trumpickas et al. 2015). Direct studies on microbial metabolism during upwelling events are needed to clarify these climate-induced changes, improve nutrient cycling models, and develop more effective management strategies.

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