Microbial community composition and dynamics in temperate, oligotrophic Flathead Lake, MT, USA

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**Introduction**

Planktonic microorganisms often form the base of limnetic food webs and catalyze biogeochemical cycles (Cotner and Biddanda 2002). Aquatic microorganisms can demonstrate vast phylogenetic and metabolic diversity (REFS), including representation among the bacteria, archaea, and eukaryotes, with metabolisms that include energy harvesting via diverse chemotrophic and phototrophic pathways (REFS). Short generation times enable microorganisms to rapidly respond to fluctuations in their environment, making these organisms potential sentinels for tracking ecosystem change. Not surprisingly, studies of the distribution, abundance, and diversity of microorganisms in lakes suggest these communities can be variable in both space and time (Wu and Hahn 2006). To date, there are relatively few studies characterizing time-scales of microorganism community composition response to seasonal changes in lake habitat structure.

Temperate, dimictic lakes undergo seasonal cycles of stratification and convective mixing due to temperature-driven changes in water density (Hutchinson and Loffler 1956). These seasonally varying stratification-mixing dynamics have important consequences for the availability of resources that support microorganism metabolism, including the flux of light and concentrations of nutrients that are available to support plankton productivity (Boehrer and Schultze 2009). Strong seasonal changes in water temperatures can have direct impacts on the individual physiologies and competitive success of planktonic microorganisms (REFS), resulting in time-varying changes to microbial community structure. Seasonal variations in lake food web structure can modify top-down processes such as predation and viral mortality, with likely impacts on the diversity and abundances of microorganisms (REFS). Hence, temperate lakes may serve as excellent case studies for characterizing time scales of microorganism community succession in response to seasonality in lake habitat structure. Consistent with this view, prior studies in temperate lakes have demonstrated seasonally-recurrent patterns in microorganism composition (Wu and Hahn 2006; Shade et al. 2007; Kara et al. 2013). However, such studies are relatively limited, in part due to the need for temporally resolved sampling spanning a full annual cycle.

Depending on the time scales of mixing relative to growth, the distributions of planktonic microbes in a well-mixed lake might be expected to exhibit vertical homogeneity. In contrast, during periods of thermal stratification, the distributions and community structure of planktonic microorganisms can partition vertically (see, e.g., Hollibaugh et al. 2001; Shade et al. 2011; Garcia et al. 2013; Linz et al. 2017) based on the availability of resources (e.g., light, nutrients) and the depth of mixing. Stratified conditions may promote vertical differentiation of niches, with each layer of within a stratified lake exhibiting relative stability in light flux, temperature, and nutrient concentrations (Yannarell and Kent 2009). Hence, the vertical arrangement of phylogenetically distinct microorganisms would be expected to vary depending on lake mixing-stratification dynamics. Such seasonal responses can provide insights into the functional roles and adaptability of microorganisms within the lake. For example, distributions of generalist microorganisms might appear largely invariant to seasonal changes, while specialists might undergo much larger seasonal fluctuations in response to habitat variability (Shade et al. 2010; Garcia et al. 2013).

Stratification and mixing influence the amount of light that organisms in the water column are exposed to, as the epilimnion can either set microorganisms at certain depths with stable light fluxes when stratified or carry microorganisms in and out of the photic zone when vertically mixed. A characteristic feature of oligotrophic ecosystems is that during periods of stratification, the epilimnion may be shallower than the photic zone (REFS). This condition forms what can be considered a ‘two-layer habitat’, where the nutrient-depleted, well-lit epilimnetic waters overlie more nutrient-enriched, but dimly-lit hypolimnetic waters (REFS). This condition often results in a subsurface chlorophyll maximum (hereafter termed Chl. Max), which resides below the epilimnion (Fee 1976; Abbott et al. 1984; Klausmeier and Litchman 2001) in the more thermally stratified hypolimnetic waters.

Stratification and mixing, in addition to affecting light and nutrient availability, influence the distribution and composition of microorganisms across depths of a lake. A well-mixed lake from surface to bottom will exhibit a single microorganism community, while stratified lakes function as multiple distinct bodies of water stacked on top of each other, enabling the creation of multiple depth-specific communities (see, e.g., Hollibaugh et al. 2001; Shade et al. 2011; Garcia et al. 2013; Linz et al. 2017). Stratified waters may allow for better niche partitioning than a fully mixed water column, as each layer of a stratified lake exhibits relatively stable conditions in terms of light, temperature, and nutrients (Yannarell and Kent 2009). Major phyla of organisms will exhibit a range of responses to these variable conditions: some may be more stable, unchanging as conditions vary in the lake, while others may be highly variable, changing seasonally or with depth. The ways that these phyla respond to changing conditions suggests their role and adaptability within the ecosystem – whether generalists who persist regardless of conditions, or specialists who undergo cycles of higher and lower abundance as conditions vary (Shade et al. 2010; Garcia et al. 2013).

Flathead Lake is a large (482 km2), oligotrophic lake in northwest Montana whose water column undergoes strong seasonal changes (Young 1935; Gaufin et al. 1976; Spencer et al. 1999; Stanford and Ellis 2002). Despite being the subject of long-term, time-series monitoring since 1977, to date there have been few studies on planktonic microorganisms that inhabit Flathead Lake and how these organisms respond to seasonal changes in lake habitat structure. The lake watershed is relatively pristine and heavily forested, maintaining high water quality throughout the year. Given the rapid pace of eutrophication occurring to lakes across the world (Scheffer et al. 2001; Prepas and Charette 2003), studies from oligotrophic lakes like Flathead provide valuable baseline information for better understanding the functioning of healthy, low-nutrient ecosystems.

We were interested in examining temporal and vertical variability associated with microorganism community composition in Flathead Lake. We expected stratification-mixing dynamics to be a major control on vertical and temporal dynamics associated with microbes in the lake. Specifically, we predicted that the composition of these microbial communities would diverge when the lake was stratified but converge when the lake was well mixed. We also expected organisms with certain specialist metabolic pathways (e.g. phototrophic microorganisms) to exhibit stronger seasonal changes and greater variability in composition than microorganisms with a more generalistic lifestyle. Over a 2 year period (2016-2018) we collected vertically-resolved samples at once- to twice-monthly intervals for subsequent amplification and sequencing of 16S rRNA genes. These sequences were used to characterize both the prokaryotic and chloroplast-derived photosynthetic eukaryote communities. We describe the emergent seasonal dynamics in the microbial community composition and describe the stability and diversity patterns of major microbial classes in time.

**Methods**

*Study site and sampling*

Sampling for this study was conducted at the long-term monitoring site termed Midlake Deep (47° 52’ 26” N, 114° 4’ 44” W), in Flathead Lake from aboard the research vessel *Jessie B*. Prior to collection of water samples, vertical profiles of water temperature, pH, conductivity, fluorescence, dissolved O2, and turbidity were measured at 1 m intervals through the upper 30 m of the lake, and 5 m intervals at depths between 30 m and 90 m using an OTT Hydrolab. Light flux was measured every 1 m for the first 5 m of the lake, then every 2 m until 32 or 34 m using an underwater spherical quantum sensor (LI-COR LI-193), standardized to a surface cosine quantum sensor (LI-COR LI-190). This profile was used to derive attenuation coefficients. In addition, the daily integrated incident PAR was derived using measurements from a shore-based weather station (47° 52’ 23” N, 114° 2’ 6” W; https://flbs.umt.edu/publicdata/) equipped with a cosine collector; the resulting 15-minute increment measurements were time-integrated to compute daily PAR incident to the surface of the lake. The attenuation coefficients and daily integrated light flux were used to calculate vertical light profiles for the entirety of the water column at Midlake Deep.

Water samples for subsequent extraction of planktonic DNA and flow cytometric quantification of microbial cell abundances were collected from 5 depths (5, 10, 50, and 90 m along with the Chl. Max) on a monthly to twice-monthly basis from September 2016 to November 2018. Water was collected using an opaque, 2.2 L Van Dorn water sampler (Aquatic Research Instruments) affixed to either a handline with demarcations at 0.5 m intervals (for 5 m, 10 m, and Chl. Max samples), or a wire spooled onto an electric winch and fed through a calibrated meter wheel (for 50 m and 90 m samples). The Van Dorn sampler was closed at discrete depths using a messenger. Water was subsampled into 1 L acid-washed, polyethylene bottles and stored (<5 hrs) in dark coolers for transport to the laboratory for processing. Water for subsequent measurements of Chl *a* concentrations were based on 0-30 m depth-integrated samples, collected using a 30 m long hose. Water was subsampled from the hose into a 20 L polyethylene carboy and stored in the dark until transport to the lab.

*Chl a concentrations and flow cytometric cell abundances*

In the laboratory, water (2 L) for determination of Chl *a* concentration was filtered onto a 47 mm diameter, glass fiber filters (nominal 0.7 m pore size). Filters were extracted in a 90% acetone solution with grinding to facilitate extraction (REFS). Chl *a* concentrations were determined spectrophotometrically, based on light absorption at XXX nm.

Abundances of phototrophic and non-pigmented picoplankton were determined using an Attune Acoustic Focusing flow cytometer. Lake water samples (2 mL) were fixed with paraformaldehyde (0.8% final concentration) and frozen in a -80°C freezer until analysis. Each sample was analyzed twice: once to quantify pigmented cells by autofluorescence, and again after the addition of the nucleic acid stain (SYBR Green I), to quantify total picoplankton abundances. Phototrophic cells (including eukaryotes and cyanobacteria) were detected via chl *a* autofluorescence, with cyanobacteria separately distinguished based on the autofluorescence of phycoerythrin. A blue (488 nm) excitation laser at 20 mW was used to stimulate fluorescence of both chl *a* and phycoerythrin, with chl *a* fluorescence detected using 640 nm longpass emission filter and phycoerythrin fluorescence detected using a R-phycoerythrin (R-PE) emission filter (574 nm and 26 nm bandwidth). SYBR-stained cells were identified using a 20 mW blue (488 nm) excitation laser and detected with a 530 nm emission filter. Non-pigmented cell abundances were calculated as the difference between the total cells (SYBR stained) and phototrophic cells.

Amplification and sequencing of 16S rRNA genes

Water samples (400-500 mL) for subsequent concentration of plankton biomass and extraction of DNA were filtered sequentially through 25 mm diameter, 3 μm pore size polycarbonate and 0.2 μm pore size polyethersulfone filters (Supor). Filters were placed in 2 mL microcentrifuge tubes containing 600 μL of a cell lysis buffer (MasterPure DNA Purification Kit; Biosearch Technologies Inc. LGC) and frozen at -80° C until DNA was extracted.

For DNA extraction, samples were thawed and 100 μL each of 0.1 mm and 0.5 mm zirconium beads were added to microcentrifuge tubes. Samples were placed in a mechanical bead beater, and agitated for 2 min, followed by DNA extraction and purification using the MasterPure DNA Purification Kit following the manufacturer’s recommendations. The concentration of DNA in each extract was fluorometrically quantified using the Invitrogen Qubit High Sensitivity dsDNA Kit (Thermo Fisher Scientific). A region of the V4-V5 of the 16S rRNA genes were PCR amplified using primers recommended by Parada et al. (2016). Amplicons were purified using the ENZA Cycle Pure Kit (Omega Bio-tek), and samples were pooled to approximately equimolar proportions in 2 libraries, then sequenced at the University of Montana Genomics Core on an Illumina MiSeq, using the MiSeq 500/PE250 v2 kit (Illumina).

*Amplicon informatic analyses*

Mothur v. 1.42.3 (Schloss et al. 2009; Schloss 2019) was used to process the sequences following the computational pipeline described in the supplemental material. Sequences were clustered to 99% sequence similarity using an abundance-based greedy clustering (AGC) method (Schloss et al. 2009; Schloss 2019), and those identified as chloroplasts after alignment and classification to SILVA v. 132 (Quast et al. 2012; Yilmaz et al. 2014) were separated from the bacterial genes for subsequent classification to different reference databases. Bacterial 16S rRNA genes were further analyzed using the TaxAss pipeline (Rohwer et al. 2018) to compare sequences classifications to both SILVA v. 132 (Quast et al. 2012; Yilmaz et al. 2014) and FreshTrain 15 (Newton and McMahon 2011; Rohwer et al. 2018). For this analysis, seqeunces were classified to 98% identity using a bootstrap p-value cut off of 80% certainty (sequences below 80% classification certainty for a given taxonomic level are identified as 'unclassified' for that taxonomic level). Those sequences identified as plastids were further classified through mothur (Schloss et al. 2009; Schloss 2019) against the PhytoREF database (Decelle et al. 2015).

After classification, the bacterial and plastid sequences were pooled for community analyses. Pooled samples were subsampled to 12,000 sequences for both size fractions. One small size fraction sample was removed for having fewer than 12,000 sequences (Chl. Max from August 7, 2017). We used the R package 'vegan' (v. 2.5.7, Oksanen et al. 2020) to subsample and calculate ordinations, multivariate statistics, and diversity metrics. The stabilities (ST) of pooled phyla were calculated following equation from Lehman and Tilman (2000) (Equation A2) as:

Where Bi represents the biomass of species i, Bj is the biomass of species j, E is the expected value, n is the number of species (indexed by i), Var is the variance function, and Cov is the covariance function. Here, we used the subsampled counts of each species ‘i’ (in this case, OTUs) in place of biomass measures. This is a reasonable substitution – the intention of equation A2 is to standardized variance and covariance to the average biomass of a community so that stability is not a function of the size of the community. Here, we were interested in the stability of the composition of each class, and so equation 2A allowed us to standardized the stability of each class’s composition to that class’s average relative abundance, allowing for comparisons across classes with a wide range of average relative abundances.

16S rRNA gene amplicon sequences are available via GenBank (SRA #XXXX). All Flathead Lake physical and biogeochemical data used for this study are available through the Flathead Lake Biological Station public data portal (https://flbs.umt.edu/publicdata/). R scripts used to generate figures and process data are available in a dedicated github repository (https://github.com/kevans27/FlatheadLake16S).

**Results**

*Seasonality in physical structure*

Over the 2 year study period, Flathead Lake demonstrated dimictic seasonal patterns (Figure 1). The surface temperature of Flathead Lake varied seasonally from summer highs of 21.5 to winter lows of 1° C (1A). The depth of the mixed layer ranged from less than 5 m during the early summer to fully mixed during the winter, while the Chl. Max sample depth ranged from 10 to 32 m, falling below the mixed layer depth during the summer. Samples from 5 m, 10 m, and Chl. Max are consistently within the well-lit portion of the lake (>X mol photon m-2 day-1), but the 50 m and 90 m samples fall below well lit zones (1B).

*Microorganism abundance*

Flow cytometric cell abundances were used to trace depth and time patterns of microorganism abundance, shown in Figure 2. Across the entire water column, non-pigmented cell count ranged from 375 to 1106 cells mL-1. Deep samples (50 m and 90 m) show little seasonality but have a maximum value of near 800 cells mL-1, lower than the maximum value in the surface (5 m, 10 m, and Chl. Max) samples. Shallow samples show more pronounced seasonality, with their maximum total cellular abundances occurring the late summer when the lake was well stratified and an early- to mid-summer minimum.

Figure 2 also shows phototrophic cellular abundances. Across the entire water column, phototrophic cell counts ranged from 2 to 180 cells mL-1. The entire water column has a phototrophic cell concentration of around 45 cells mL-1 at the end of the well mixed season (March and April). The concentration dropped off in the deep (50 m and 90 m) samples immediately after stratification but continued to increase through May in the shallower samples. The shallowest samples (5 m and 10 m) showed a pronounced two-peak annual pattern of phototrophic cellular abundance in both years, with maximum values of around 75 cells mL-1 occurring in both the early stratification (May and June) and late stratification (September and October). Notably, during mid stratification (July and August), the abundance of phototrophic organisms in the surface water dropped down to the same, minimum level observed when the water column was fully mixed (December and January). The Chl. Max demonstrated unique seasonal patterns with a maximum phototrophic abundance (180 cells mL-1) during September of 2017, which was a different time of year than the maximum phototrophic abundance values of any other depth. This pattern was not observed in 2018, but this may be due to a lack of temporal resolution because of missing samples at the Chl. Max.

*Seasonality in the similarity of the communities across depths*

To query mixing patterns of the lake, in addition to temperature profiles, we studied the similarity in the 16S rRNA community composition across depths from each sampling trip. Figure 3 shows two NMDS plots based on Jaccard distances, one for each size fraction (small, 0.2 – 3 um, and large, >3 um), for all samples collected. Both size fractions exhibit the same general pattern, although more pronounced in the large samples: the deep samples are clustered and show little seasonality, while the shallower samples diverge from the deep samples during stratified periods but are clustered with the deep samples when well mixed (December through April). Thus, Figure 3 shows patterns consistent with a classic, monomictic community.

Figure 4 uses Jaccard dissimilarity values to illustrate the relationship between community composition and mixing patterns. In this figure, the community composition of samples from 10 m, the Chl. Max, 50 m, and 90 m were each compared to the community from the 5 m sample of the same sampling trip. Higher values of Jaccard dissimilarity reflect more dissimilar communities between that depth and the 5 m sample. As expected, when the lake is stratified, the samples diverge from 5 m, with samples closer in depth to 5 m retaining lower values of Jaccard dissimilarity than deeper samples. During the fully mixed periods, Jaccard dissimilarities from all depths collapse on top of one another, becoming equally and minimally dissimilar from 5 m. This does not necessarily mean that all depths are dissimilar to 5 m in the same way, just that the dissimilarity value is equal across depths. Notably, in 2017, dissimilarity across depths collapsed in December and May, but the depths diverged mildly during the winter, with the deepest samples becoming more dissimilar from 5 m than the shallowest samples. In contrast to Figure 3 but consistent with Figure 1, this suggests mild, inverse stratification during the winter of 2017.

Figure 5 uses Shannon’s Diversity Index to illustrate mixing. Each panel represents a different date of sampling, with the mixed layer depth, shown in Figure 1, shaded in blue. Samples within the mixed layer generally show similar diversity index values, which is well illustrated during the winter samples of 2018 when the entire water column is vertically mixed. Diversity values appear generally lower when the lake is well stratified (May through October), particularly in the mixed layer samples.

*16S community composition*

Figure 6 shows the prokaryotic class community composition for each sample. The top 8 most abundant classes account for an average of 85% of the community within each sample*.* The top 8 most abundant classes are all classes of Bacteria, while Archaea account for an average of 0.07% of each sample. The small size fraction (Panel 1A) was nearly always dominated by Actinobacteria in the surface water, and the relative contribution of Actinobacteria decreased with depth. Actinobacteria were far less abundant in the large size fraction (1B), which appeared dominated by Oxyphotobacteria in the shallow samples and unclassified or low abundance classes in the deeper samples (“Other Prokaryotes”).

The strength of seasonality varies with depth for both small and large size fractions, with both size fractions showing little seasonality in the deepest samples (50 m and 90 m). In the small size fraction (1A), the shallower samples (5 m, 10 m, and Chl. Max) showed increases in Actinobacteria during the late summer (July through September), increases in Gammaproteobacteria and Alphaproteobacteria during the mid-summer (June through August), and increases in Acidomicrobiia during the winter (December through May). In the large size fraction, shallow samples showed pronounced increases in Oxyphotobacteria during the summer (July through October) and increases in Planctomycetacia during the winter (November through April).

*Habitat preference, diversity, and stability of organisms*

Figure 7 shows the differential abundance of the top 10 most abundant classes of organisms, including photosynthetic eukaryotes, in terms of depth preference (shallower or deeper) and surface temperature (warmer and cooler lake surfaces), which is used as a proxy for mixed status of the lake. The Oxyphotobacteria and Chrysophyceae appeared more abundant in shallower water, which is expected for organisms utilizing photoautotrophy. This is contrasted by the Phycisphaerae, which appear most often in deep, cool water. Bacteroidia showed little to no differential abundance in terms of depth or surface temperature, suggesting generalist behavior that is uninfluenced by seasonal effects on the water column. Actinobacteria appear to be more abundant in warmer, shallower water, particularly in the large size fraction (Figure 7B), although this preference appears weaker in the small size fraction (7A).

The Oxyphotobacteria and Phycisphaerae, two classes with pronounced environmental effects on relative abundance shown in Figure 7, are used to discern the mixed status of the lake, shown in Figure 8. Figure 8 shows the relative abundance of the two classes in both size fractions through depth and time, with the mixed layer overlaid. Interestingly, both classes of organisms increase in relative abundance during the early spring (March and April), despite the entire water column appearing to be mixed. This suggests that our periodic temperature sampling is missing some element of lake stratification and mixing patterns: either the water column is mixing so slowly that these organisms can grow at their preferred depth before being mixed throughout the water column, or the water column is undergoing periods of full vertical mixing alongside periods of stratification.

Figure 7 showed variability in the relative abundance of the top 10 most abundant classes through depth and time, but there was also variation at the OTU level within each class. Figure 9 shows the relationship between each class’s diversity at the OTU level and stability (temporal and depth-related changes of community composition between samples). Figure 9 relates the average diversity, measured by Shannon’s Diversity Index, evenness, and richness, with the stability of the community composition across all samples for the top 10 most abundant classes.

In the small size fraction, Actinobacteria appear remarkably diverse, with the highest average values of richness (Figure 9C) and Shannon’s Diversity (9A). This highly diverse class is also the third most stable class of the small size fraction. Actinobacteria are sharply contrasted by the Oxyphotobacteria, which the lowest evenness value (9E), the second lowest Shannon’s Diversity Index (9A) and the second lowest stability. The least stable class in the small size fraction is the Phycisphaerae, which have a very low average relative abundance. The small Actinobacteria and Oxyphotobacteria, while both being relatively abundant classes, appear to highlight two extremes of the stability vs. diversity continuum, with a highly diverse, stable community of Actinobacteria and a highly uneven, unstable community of Oxyphotobacteria.

A similar, although less extreme, pattern is observed in the large size fraction. The Oxyphotobacteria, again, represent a low diversity (9B), low stability class, while the Bacteroidia represent a higher diversity, higher stability class. The only two classes less diverse than the Oxyphotobacteria in the large size fraction are both classes of photosynthetic eukaryotes (Bacillariophyta and Chrysophyceae).

*Composition of putative phototrophic organisms*

The community composition of the unstable, uneven phototrophic organisms is plotted in Figure 10, which shows the relative abundance (standardized to the total Cyanobacteria and Ph. Euks count per samples) for each sample in both size fractions. The small size fraction (Figure 10A) appears to have more Cyanobacteria than Ph. Plastids, while the large size fraction (10B) generally has more Ph. Plastids than Cyanobacteria. In both size fractions, the Cyanobacteria appear dominated by a single lineage (Cyanobiaceae), with occasionally substantial Microcystaceae and Pseudanabaenaceae communities. The Ph. Plastids are much more diverse than the Cyanobacteria in both size fractions, showing a general dominance of Bacillariophyta in the large size fraction and Cryptophyceae in the small size fraction, with large variances between samples. In the surface water (5 m, 10 m, and the Chl. Max) of both size fractions, Chrysophyceae appear strongly seasonal, occasionally becoming the largest community in the large size fraction in June, July, and August.

The Cyanobiaceae dominance of the Cyanobacteria is remarkably uneven, shown in Figure 11, which plots the relative abundance of each Cyanobiaceae OTU. The OTUs are named according to their average relative abundance across all samples: Cyanobiaceae OTU2 and OTU4 are the 2nd and 4th most abundant prokaryotic organisms in Flathead Lake, on average. This is despite their being putative photosynthetic organisms and having large seasonal changes in relative abundance, suggested by the Oxyphotobacteria in Figures 7, 8, and 9. OTU2 and OTU4 account for an average of 62% of the Cyanobacteria of all samples, but have distinct seasonal patterns, with OTU2 becoming more abundant during the winter and early stratification (January through July), and OTU4 becoming more abundant during the mid- to late-stratification (August through November). The Cyanobiaceae dominate the Cyanobacteria of the lake but are extremely uneven.

*Actinobacteria community composition*

The unevenness of Cyanobiaceae in Figure 11 is contrasted by the stable, diverse community of Actinobacteria shown in Figure 12. In both size fractions, the relative composition of the Actinobacteria changes very little through the year or even across depths, apart from acl-A6 (yellow), which shows an increase in relative abundance in the late summer. Note also that the dark green bar (‘Other Actinobacteria’) represents the Actinobacteria tribes which are not the top 8 most abundant, and those less abundant tribes combined are more abundant than the most abundant single tribe, acl-B1, suggesting a relatively even community. The temporal and depth stability of the Actinobacteria community in Figure 11, supported by stability of the Actinobacteria composition shown in Figure 7, suggests a generalist behavior of the class.

Chart, histogram

Description automatically generated

**Figure 1**: Temperature (A) and light profile (B) contour plots of Flathead Lake at Midlake Deep. Discrete, black points represent the depth of the Chl. Max sampled on those dates, while the continuous gray line represents the mixed layer depth. Data obtained from the Flathead Lake Biological Station public data portal (https://flbs.umt.edu/publicdata/).

Chart, diagram

Description automatically generated

**Figure 2**: Flow cytometric cell counts for non-pigmented (pink) and phototrophic (green) cells for the duration of the 16S rRNA gene amplicon sampling. Non-pigmented cell count values are shown on the left axis while phototrophic cell count values are shown on the right axis. Note that the phototrophic cells at the Chl. Max have a different scale than the phototrophic cells at other depths by a factor of two. Gray shading represents the time between the fall and spring mixing events in Flathead Lake.

Chart, scatter chart

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**Figure 3**: NMDS plot based on Jaccard distances for all samples in both the small (upper panel) and large (lower panel) size fractions. Samples are colored by month in a cyclic color scheme (color similarity reflects the annual cycle, with the colors for December and January being as similar as June and July) and the point shape represents the sampling depth, with filled points representing samples that were typically within the photic zone and empty shapes representing samples below the photic zone.

Chart, scatter chart

Description automatically generated

**Figure 4**: Jaccard dissimilarity values through time calculated for each depth of each sampling date relative to the 5 m sample from the same date. Lower values suggest more closely related communities while higher values represent more distinct communities. Gray shading represents the time between the fall and spring mixing events in Flathead Lake.

Chart

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**Figure 5**: Shannon’s Diversity Index for each sample, plotted by date (column), size fraction (row), and depth of sample (order within column). The blue shaded areas represent samples within the mixed layer for that date according to the mixed layer depth calculated in Figure 1. Vertical dashed, black lines between samples separate years.

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**Figure 6**: Prokaryotic community composition for the large (left) and small (right) size fractions through time and by depth, with the shallowest (5 m) samples in the top panels and the deepest (90 m) in the bottom panels. Each color represents a different prokaryotic class and white bars represent missing or removed samples. The light green bar represents prokaryotic classes not included in the top 8 most abundant classes. The legend is ordered from most to least abundant on average.

Diagram

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**Figure 7**: Differential abundance for top 8 classes in each size fraction with depth effects plotted on the x axis and surface temperature effects on the y. Gray dashed lines represent the axes where depth (in the case of the vertical line) or surface temperature (in the case of the horizontal line) would have no influence on the differential abundance of a class.

A picture containing text, electronics, display

Description automatically generated

**Figure 8:** Contour plot demonstrating Oxyphotobacteria (top) and Phycisphaerae (bottom) relative abundance in the small (left) and large (right) size fractions for the duration of our study period. The color bar on the right is scaled in terms of relative abundance as a percentage, and the black line represents the mixed layer depth.

**Diagram

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**Figure 9**: Stability vs. diversity metrics for the top 10 most abundant classes in each size fraction. Each class is labeled, and the size of the circle corresponds to its average relative abundance across all samples. Blue classes are prokaryotes while red classes are photosynthetic eukaryotes. Stability (ST) is calculated using equation A2 from Lehman and Tilman (2000), and it includes all samples, regardless of depth or date of sampling.

Chart

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**Figure 10**: Primary producer community composition for the small (left) and large (right) size fractions from all samples. This includes by the Cyanobacteria, displayed at a lineage level, and the Ph. Plastids, displayed at a class level. White columns represent missing or removed samples. The light green color represents other Cyanobacteria, which were not in the top 7 most abundant primary producers, and the dark red color represents other Ph. Euks. Bars are standardized to the primary producer abundance of each sample rather than the entire sequenced community.

Chart

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**Figure 11**: Cyanobiaceae OTUs for both size fractions (small on left and large on right) for all samples. White columns represent missing or removed samples. The light blue color represents all other Cyanobiaceae OTUs which were below the top 7 most abundant. Bars are standardized to the Cyanobiaceae abundance of each sample rather than the entire sequenced community.

Chart

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**Figure 12:** Composition of Actinobacteria tribes for the small (left) and large (right) size fractions through time and by depth, with the shallowest (5 m) samples in the top panels and the deepest (90 m) in the bottom panels. Each color represents a different Actinobacteria tribe and white columns represent missing or removed samples. The columns are standardized to the total Actinobacteria 16S rRNA gene counts rather than the total community. The dark green bar represents all Actinobacteria tribes that are not included in the 8 most abundant tribes. The legend is ordered from most to least abundant on average.

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