# Methods

**Table 1. Location and characteristics of study sites.** The lakes included in this time series are small, humic bog lakes in the boreal region near Minocqua, Wisconsin, USA. They range in depth from 2 to 21.5 meters and encompass a range of water column mixing frequencies (termed regimes). Dimictic lakes mix twice per year, typically in fall and spring, while polymictic lakes can mix more than twice throughout the spring, summer, and fall. Meromictic lakes have no recorded mixing events. pH was measured in 2007, while nutrient data was measured in 2008. When two values are present in a single box, the first represents the epilimnion value and the second represents the hypolimnion value.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Forestry Bog | Crystal Bog | North Sparkling Bog | West Sparkling Bog | Trout Bog | South Sparkling Bog | Hell’s Kitchen | Mary Lake |
| ID | FB | CB | NS | WS | TB | SS | HK | MA |
| Depth *(m)* | 2.0 | 2.5 | 4.5 | 4.6 | 7.0 | 8.0 | 19.3 | 21.5 |
| Surface area *(m2)* | 1300 | 5600 | 4700 | 11900 | 10100 | 4400 | 30000 | 12000 |
| Mixing regime | Polymictic | Polymictic | Dimictic | Polymictic | Dimictic | Dimictic | Meromictic | Meromictic |
| GPS coordinates | 46.047776, -89.651248 | 46.007639, -89.606341 | 46.004819, -89.705214 | 46.004633, -89.709082 | 46.041140, -89.686352 | 46.041140, -89.709082 | 46.186674, -89.702510 | 46.250764, -89.900419 |
| Years sampled | 2007 | 2007, 2009 | 2007, 2008, 2009 | 2007 | 2005, 2007, 2008, 2009 | 2007, 2008, 2009 | 2007 | 2005, 2007, 2008, 2009 |
| pH | 4.97, 4.85 | 4.49, 4.41 | 4.69, 4.80 | 5.22, 5.14 | 4.60, 4.78 | 4.46, 4.94 |  | 5.81, 5.72 |
| Dissolved inorganic carbon (ppm) |  | 0.69, 1.72 | 1.12, 2.31 |  | 1.73, 4.47 | 1.97, 6.42 |  | 5.54, 12.38 |
| Dissolved organic carbon (ppm) |  | 15.47, 13.6 | 10.05, 10.40 |  | 19.87, 20.58 | 12.40, 21.92 |  | 20.63, 67.10 |
| Total nitrogen (ppb) |  | 620.57, 846.00 | 629.09, 809.45 |  | 737.71, 1121.00 | 813.88, 1498 |  | 1332.57, 3652.38 |
| Total phosphorus (ppb) |  | 30.00, 38.86 | 78.00, 135.45 |  | 50.57, 53.25 | 48.63, 69.14 |  | 78.00, 303.50 |
| Total dissolved nitrogen (ppb) |  | 1290.19, 490.13 | 442.39, 586.56 |  | 582.5, 820.21 | 451.63, 1179.21 |  | 1024.5, 3220.14 |
| Total dissolved phosphorus (ppb) |  | 84.25, 14.88 | 70.22, 22.67 |  | 34.5, 31.57 | 16.25, 18.29 |  | 71.13, 228 |

# Results

## Overview of community composition

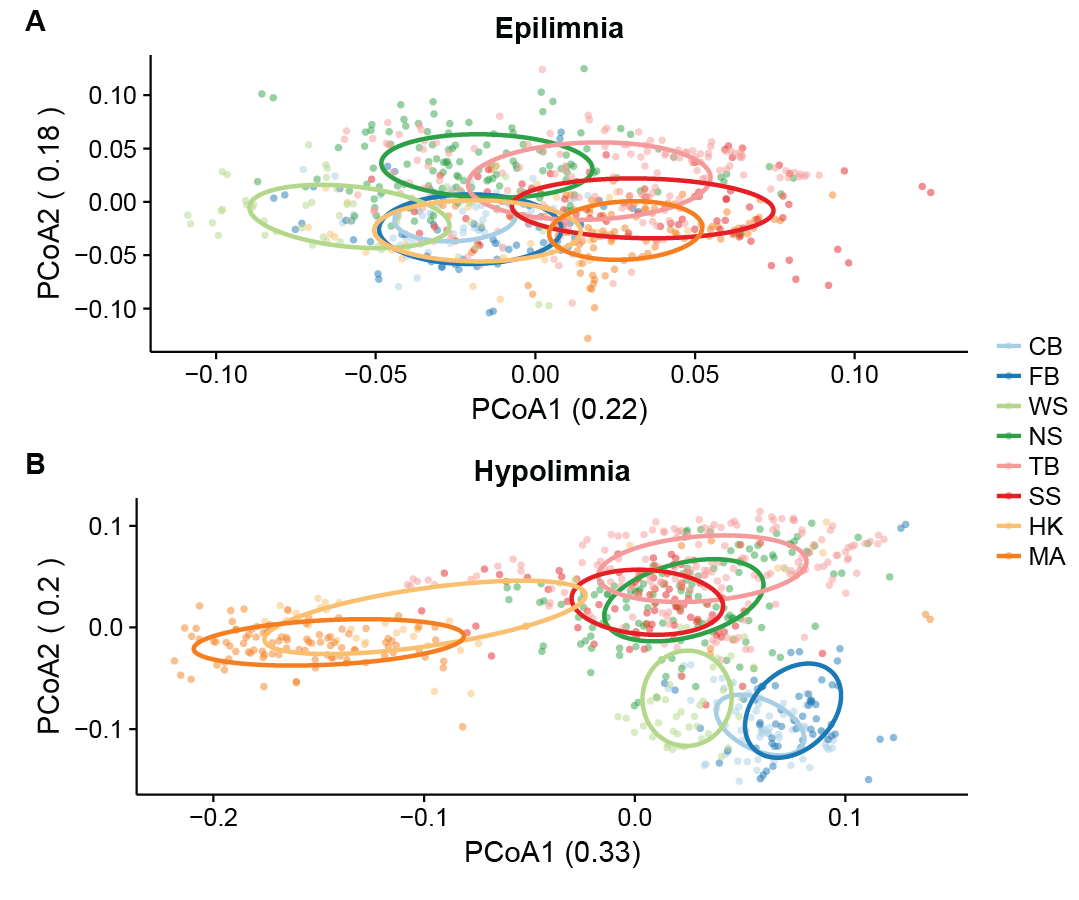
A time series of 16S amplicon data recovered from 1,387 samples was used to investigate bacterial community composition over time and across lakes. A total of 8,795 OTUs were detected. As is typical for most freshwater ecosystems, Proteobacteria, Actinobacteria, Bacteroidetes, and Verrucomicrobiawere the most abundant phyla (Figure S1). Within these phyla, OTU abundance was highly uneven. For example, much of the abundance of *Proteobacteria* could be attributed to OTUs belonging to the well-known freshwater groups *Polynucleobacter* and *Limnohabitans,* and the freshwater clade acI contributed disproportionately to the observed abundance of Actinobacteria. Like many microbial communities, unevenness was a recurring theme in this dataset, which had a long rare tail of OTUs and trends driven largely by the most abundant OTUs (1, 2). Trimming of rare taxa did not impact the clustering observed in ordinations, such as those present in Figure 2, even when taxa observed less than 1000 times were removed.

## Community richness

We hypothesized that disturbance frequency, indicated by mixing regime, determines biodiversity levels. Observed richness was calculated for every sample at the OTU level, and samples were aggregated by lake and layer. Hypolimina were on average more rich than epilimnia (Figure 1, Table S1). Significant differences in richness between lakes were detected. For both layers, polymictic lakes had the fewest taxa, meromictic lakes had the most taxa, and dimictic lakes had intermediate numbers of taxa. This does not support our initial hypothesis that intermediate disturbance leads to the greatest amount of biodiversity. Instead, richness appears to increase with depth. We note that many variables covary with lake depth, such as mixing regime, volume of integrated water column, dissolved carbon concentrations and total nitrogen concentration. Thus, it is not clear which factor is responsible for the observed trend in richness.

## 

**Figure 1. Richness by layer and lake.** Lakes on the x axis are arranged by depth (see Table 1 for lake abbreviations and depth measurements). Lakes CB, FB, and WS are polymictic, lakes NS, TB, and SS are dimictic, and lakes HK and MA are meromictic. Colored bars above each plot represent significant differences in richness between lakes, with each colored bar matching the color of a lakes boxplot. For example, in Panel A, the boxplot for CB has the colored bars matching FB, NS, TB, SS, HK, and MA above it. This indicates that it is significantly different from these lakes, but not significantly different from the missing colored bar, WS.



**Figure 2.** Weighted UniFrac distance was used to perform principal coordinates analysis on epilimnion (A) and hypolimnion (B) samples. In both layers, samples cluster significantly by lake and mixing regime as tested using PERMANOVA. (See Table 1 for lake abbreviations; CB, FB, and WS are polymictic, NS, TB, and SS are dimictic, HK and MA are meromictic). Ellipses indicating the clustering of each lake were calculate based on standard error using a 95% confidence interval. Differences in bacterial community composition between lakes and mixing regimes are more pronounced in hypolimnia than epilimnia.

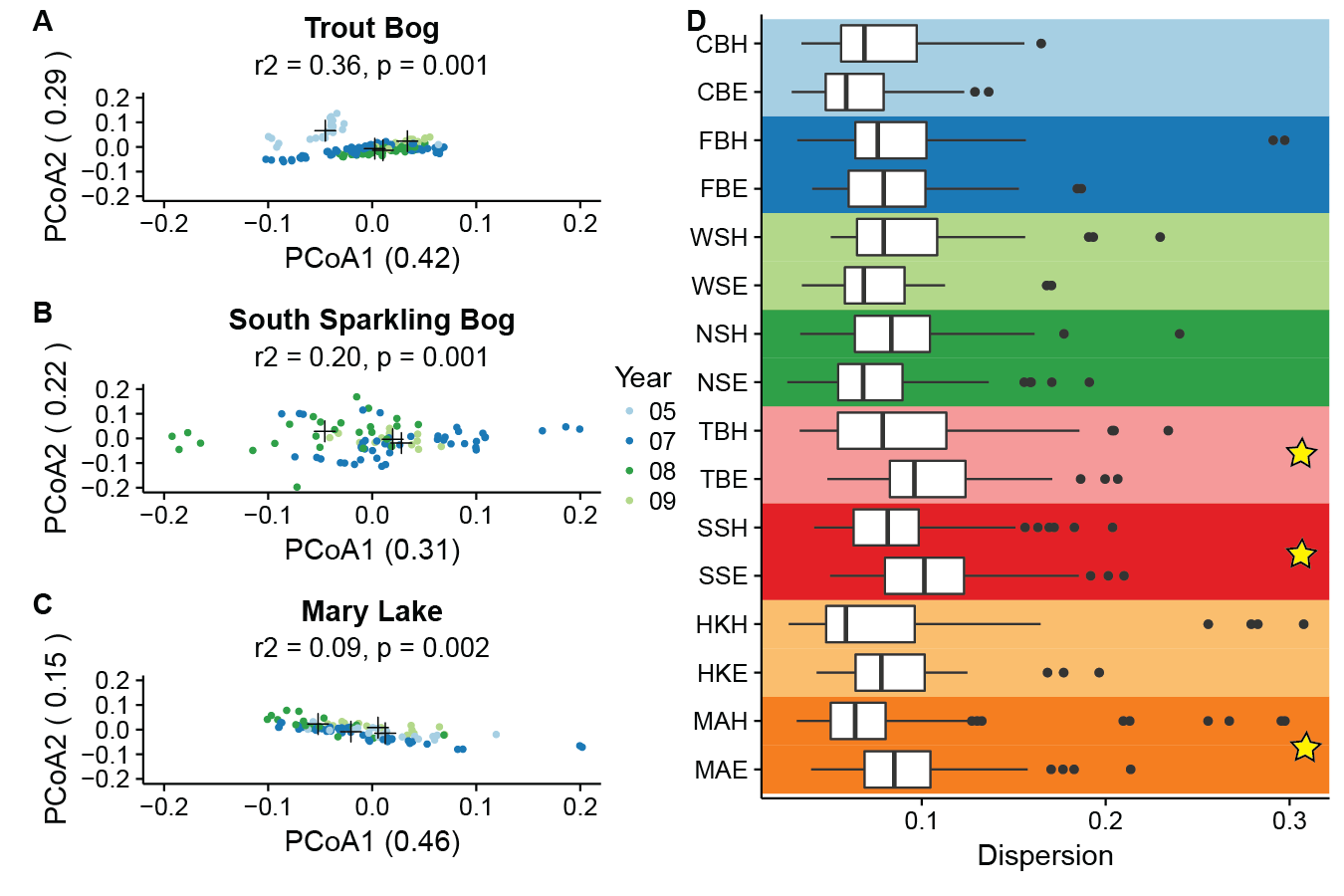
## Clusters of community composition

When differences in community composition were quantified using weighted UniFrac distance and visualized using principal coordinates analysis, several trends emerged. The large number of samples precluded much interpretation using a single PCoA, but sample clustering by layer, mixing regime, and lake was evident (Figure S2). Thus, we also examined PCoA for single lakes (both layers). Communities from the epilimnion and hypolimnion layers were significantly distinct from each other at p < 0.05 in all lakes except Forestry Bog (FB) (p = 0.10) (Figure S3).

Within layers, mixing regime was the next factor explaining differences in community composition (Figure 2). Clustering by mixing regime was significant by PERMANOVA in both epilimnia and hypolimnia samples (r2 = 0.20 and r2 = 22, respectively, and p = 0.001 in both groups). Lake was a strong factor explaining community composition, with significant cluster in epilimnia (p = 0.001, r2 = 0.34) and hypolimnia (p = 0.001, r2 = 0.49). As previously mentioned, depth and mixing regime in lakes co-vary with several other environmental variables such as dissolved carbon and nitrogen concentrations.

## Variability and dispersion

While community composition was distinct by layer, lake, and mixing regime, there was still variability in community composition over time. Each year in each lake had a significantly different community composition, indicating interannual variability in the community composition (Figure 3a-c, Figure S4). We found no evidence of repeating seasonal trends during the stratified summer months in these lakes. Likewise, we examined the abundance trends of the most abundant individual OTUs and they did not seem to repeat each year, even when abundances in each year were normalized using z-scores (Figure S5).



**Figure 3. Internannual variability and dispersion by lake.** Principal coordinates analysis using weighted UniFrac as the distance metric was used to measure the amount of interannual variation in lake hypolimnia (A-C). Black crosses indicated the centroid for each year. All hypolimnia showed significant clustering by year by PERMANOVA. Six outliers in Mary Lake from 2007 are not shown, as their coordinates lie outside the range specified for consistency between plots; these points were included in the PERMANOVA significance test. Panel D shows dispersion of each lake and layer in a PCoA including all samples (Lake abbreviations found in Table 1; E indicates epilimnion and H indicates hypolimnion; 6 outliers with distances from the centroid greater than 0.45 were removed). Stars indicate significant differences between layers at p < 0.05 by PERMADISP with a Bonferroni correction for multiple pairwise comparisons. Layers were signficantly different in TB, SS, and MA. No significant differences in dispersion between layers in the polymictic lakes (CB, FB, and WS), meromictic lake HK, or NS, a dimictic lake with an additional artificial mixing event.

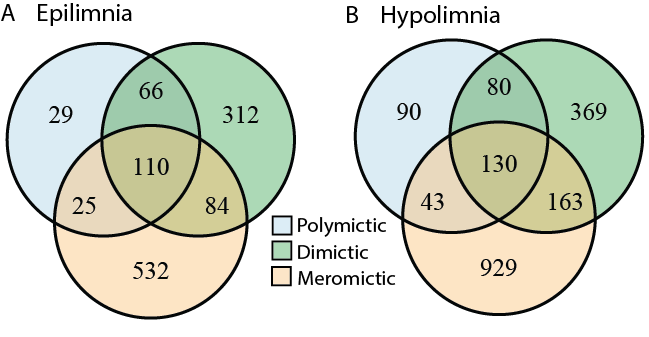
Varibility can also be assessed by measuring the dispersion of groups in PCoA. Dispersion is the distance of each point from the centroid of a group on an ordination plot. This analysis showed that layers had significantly different degrees of dispersion in two of the dimictic lakes (Trout Bog and South Sparkling Bog) and a meromictic lake (Mary Lake) (Figure 3d). Two outliers in Mary Lake were removed; these dates showed different community compositions dominated by few taxa, likely the result of a bloom event. Dispersion was not significantly different in the polymictic lakes, dimictic North Sparkling Bog, and meromictic Hell’s Kitchen. North Sparkling Bog likely did not have significant differences between layers because it was artificially mixed during stratification in 2008 (3), essentially making it polymictic for one year. Hell’s Kitchen has only one year of sampling, which may prevent detection of significance. When dispersion between layers was significant, the epilimnion was on average more dispersed than the hypolimnion, indicating higher variability. This is consistent with previously published results, and confirms that epilimnia are more variable than hypolimnia.

## The core community of bog lakes

One of the goals of this study was to determine the core bacterial community of bog lakes in general, and of specific types of lakes. Our previous analyses in Figure 2 showed that community composition was distinct in each layer and lake, while the data presented in Figure 3 showed substantial variability in community composition within the same lake and layer. Still, rarefaction curves generated for the entire dataset and for each layer begin to level off, suggesting that we have sampled the majority of taxa found in our study sites (Figure S6). To identify the taxa that comprise the bog lake core community, we defined “core” as being present in 90% of a group of samples, regardless of abundance. Four OTUs met this criteria for all samples in the dataset: OTU0076 (bacI-A1), OTU0097 (PnecC), OTU0813 (acI-B2), and OTU0678 (LD28). These taxa were therefore also core in both epilimnia and hypolimnia. Additional core taxa in epilimnia also included OTU0004 (betI), OTU0184 (acI-B3), OTU0472 (Lhab-A4), and OTU0522 (alfI-A1), while additional hypolimnia core taxa included OTU0042 (Rhodo), OTU0053 (unclassified Verrucomicrobia), and OTU0189 (acI-B2).

We performed the same core analysis on groups created by combining OTUs with the same tribe level classification (defined by 95% nucleotide similarity in the full length16S region), since in some tribes, OTUs were specific to certain sites. Notably, some OTUs were endemic to specific lakes, even though their corresponding tribe was found in multiple lakes/layers. OTUs not classified at the tribe level were not included. Results were similar to those at the OTU level. Core tribes of all samples included bacI-A1, PnecC, acI-B2, and LD28, but also betIII-A1 and acI-B4. In epilimnia, the core tribes were bacI-A1, PnecC, betIII-A1, acI-B3, acI-B2, Lhab-A4, alfI-A1, LD28, and acI-B4, while in hypolimnia, they were Rhodo, bacI-A1, PnecC, betIII-A1, acI-B2, and acI-B4. These results show that despite lake-to-lake differences and interannual variability, there are bacterial taxa that are consistently present in bog lakes.

Principal coordinates analysis in Figure 2 suggested that samples clustered by mixing regime as well as by lake and layer. To better visualize the overlap in community composition among lakes with different mixing regimes, we began by making Venn diagrams of OTUs shared and unique to each mixing regime (Figure 4). In this analysis, an OTU needs to have appeared in only one sample at any abundance to be considered present. In both epilimnia and hypolimnia, meromictic lakes had the greatest numbers of unique OTUs while polymictic lakes had the least, consistent with the differences in richness between lakes (Figure 1). Meromictic and dimictic lakes shared the most OTUs in both layers, followed by dimictic and polymictic lakes, and then by meromictic and polymictic lakes. This suggests that there is a gradient of shared community membership across mixing frequencies.



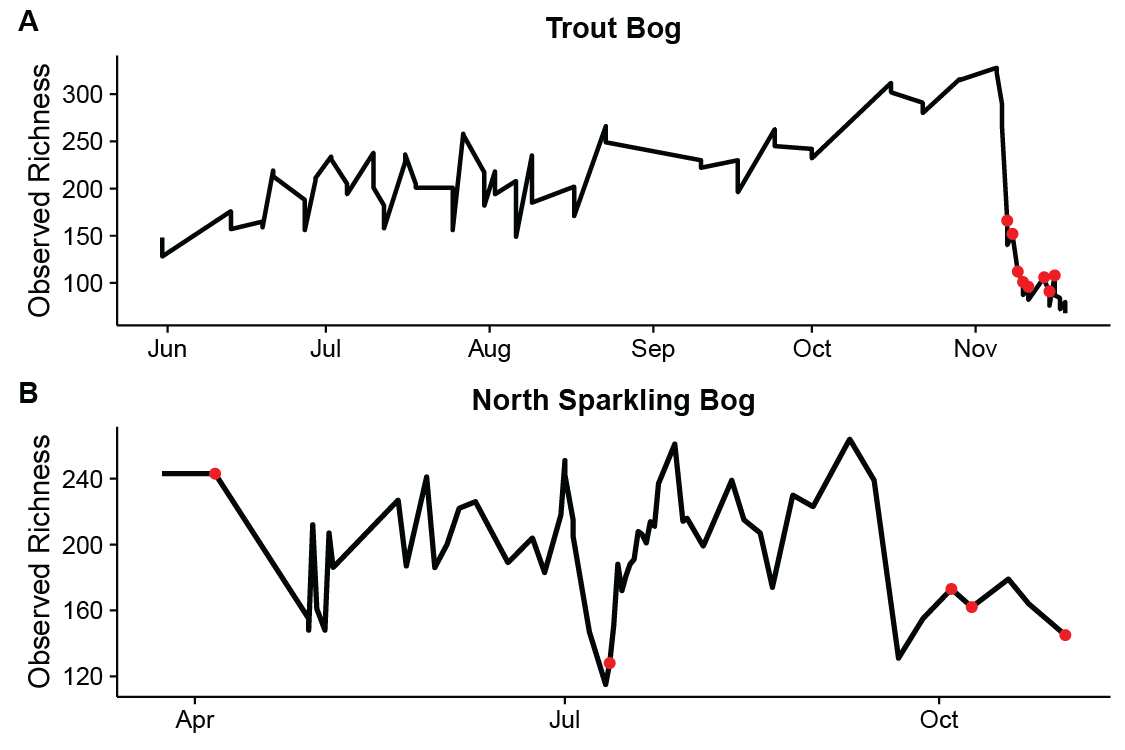
**Figure 4. Numbers of unique and shared OTUs by mixing regime.** To better understand how shared community membership differs by mixing regime, we quantified the number of shared and unique OTUs in each category. An OTU needs only to appear in one sample at any abundance to be considered present in a category. We found that in both layers, meromictic lakes have the greatest numbers of unique OTUs and polymictic lakes have the least. Meromictic and dimictic lakes share the most OTUs, while meromictic and polymictic lakes share the least. Dimictic lakes share more OTUs with meromictic lakes than with polymictic lakes.

We next used indicator analysis to identify the taxa unique to each mixing regime. Indicator analysis is a statistical method used to determine if taxa are found significantly more often in certain pre-determined groups of samples than in others. In this case, the groups were defined by mixing regime, and normalization was applied to account for different numbers of samples in each group. OTUs were combined at every taxonomic level, and all taxonomic levels were run in the indictor analysis at once to account for differences in the ability of these levels as indicators (for example, the order Actinomycetales is a stronger indicator of polymictic lakes than the phylum Actinobacteria). An abundance threshold of 500 reads was imposed on each taxonomic group. The full table of results from the indicator analysis are available in the supplemental material, while a few indicator taxa of interest are highlighted here.

The clade acI is a ubiquitous freshwater group, with specific lineages and tribes showing a preference for bog lakes in previous studies (4, 5). Our dataset shows a further distinction of acI by mixing regime in epilimnia; acI-A tribes are found predominantly in meromictic lakes, with exception of Phila, which is an indicator of polymictic lakes. Tribes of acI-B, particularly OTUs belonging to acI-B2, are indicators of dimictic lakes. Methylophilales, a putative methylotroph, is also an indicator of dimictic lakes, as is putative sulfate reducer Desulfobulbaceae. The phyla Planctomyces, Omnitrophica (formerly OP3), OP8, and Verrucomicrobia found more often in meromictic lakes, as are putative sulfate reducers Syntrophobacterales and Desulfobacteraceae. Indicators of polymictic lakes include ubiquitous freshwater groups such as Limnohabitans, Polynucleobacter (PnecC), betI-A, and verI-A. These indicator taxa likely reflect the environmental conditions unique to each mixing regime.

## Community composition during mixing events

One of the reasons bog lakes were chosen for this study was their fast transitions from mixed to stratified and back to mixed. Despite the transient nature of mixing events, two were captured in this dataset. One is a natural fall mixing event in dimictic Trout Bog in 2007, and the other is the artificial mixing of dimictic North Sparkling Bog in the summer of 2008 (6). These two events give us a unique perspective on how the bacterial community changes in response to mixing events.

**Figure 5. Richness over time during mixing events.** In both panels, the black line traces the number of OTUs observed at each time point, and the red dots indicate dates on which the water column was uniform in temperature (mixed). Sharp decreases in richness are observed during both the fall mixing in Trout Bog, 2007 (A) and the artificial mixing in July in North Sparkling Bog, 2008 (B). Transient mixing dates in the fall of 2008 in North Sparkling Bog also show lower richness.

Observed richness was traced over time in the years of both mixing events. This analysis revealed sharp decreases in both lakes. In the case of the Trout Bog fall mixing, richness remained low until the end of the sampling season. In North Sparkling Bog, richness quickly returned to the levels observed before the artificial mixing. This confirms previous results indicating resilience in the freshwater bacteria community (7), and suggests that while mixing acts as a bottleneck that only certain taxa can tolerate, but that a source of diversity exists that allows fast re-establishment of the community.

If a reduced number of taxa can survive or even thrive during mixing events, which taxa are they? In Trout Bog hypolimnion in 2007, the top ten most abundant OTUs belonged to groups such as PnecC, Methylococcales, bacI-A1, acI-B2, Methylophilales, Acetobacteraceae, and acV-A2. The North Sparkling Bog hypolimnion mixed dates shared PnecC, Methylococcales, bacI-A1, acI-B2, and acV-A2, suggesting that these are the taxa selected for by the conditions of a mixing event. However, North Sparkling Bog also had high abundances of acI-B3, acI-B4, and Lhab-A4, but did not contain Acetobacteraceae and Methylophilales in its top ten most abundant OTUs during mixed dates.

# Discussion

The North Temperate Lakes - Microbial Observatory dataset is a comprehensive 16S amplicon survey spanning four years, eight lakes, and two depths. We found that multiple years of sampling were necessary to describe the community of bog lake ecosystems. Richness and membership in these communities were structured by layer, mixing regime, and lake. We identified specific bacterial taxa present throughout the dataset, as well as taxa endemic to certain depths or mixing regimes. Mixing events were associated with reduced richness and an increase in the proportion of certain taxa. High levels of variability were detected in this dataset; each year in each lake harbored a unique bacterial community. Our results emphasize the importance of multiple sampling events to assess full bacterial community membership and variability.

The bog lakes in this study have been model systems for freshwater microbial ecology for many years. Early studies used Automated Ribosomal Intergenic Spacer Analysis (ARISA), a fingerprinting technique for identifying unique bacterial taxa in environmental samples (8). Our research built upon these studies and added information about the taxonomic identities of bacterial groups. For example, persistent and unique bacterial groups were detected in the bog lakes using ARISA (9); using 16S amplicon sequencing, we determined that these groups are the ubiquitous freshwater bacteria LD28, acI-B2, PnecC, and bacI-A1. Differences in richness and community membership were previously detected in Crystal Bog, Trout Bog, and Mary Lake, three sites representative of the three mixing regime categories of polymictic, dimictic, and meromictic (9). Our data supported these results and suggest that these trends are indeed linked with mixing regime, as we included multiple lakes of each type in this study.

We also supported previous research on the characteristics of bacterial communities in the epilimnion and hypolimnion, and the impacts of lake mixing on these communities. We confirmed that epilimnia tended to be more variable than hypolimnia, potentially due to increased exposure to climatic events (9). Mixing was disruptive to both epilimnion and hypolimnion communities, selecting for only a few taxa that can thrive during this disturbance, but quickly recovering diversity (7, 3). Comparing richness between lakes of different mixing regimes did not support the intermediate disturbance hypothesis, our initial inspiration for the collection of this dataset; rather, the least mixed lakes had the most diverse communities. As many variables are co-dependent with mixing regime (such as depth, volume of integrated water column, dissolved carbon concentrations and total nitrogen concentration), it is not clear which variables are driving this trend.

We were not able to detect seasonal trends in bog lakes in our multiple years of sampling. While seasonality in marine and river systems has been well-established by our colleagues, previous research on seasonality in freshwater lakes has produced mixed results (10–13). Nelson identified distinct, repeatable community types in his seminal paper on seasonality in alpine lakes, but noted that stratified summer communities were distinct each year (14). Seasonal trends were detected in a time series from Lake Mendota similar to ours, but summer samples in Lake Mendota were more variable then those collected in other seasons (15). In the previous ARISA-based research on the bog lakes included in our dataset, community properties such as richness and rate of change were consistent each year, and the phytoplankton community hypothesized to drive seasonal trends in the bacterial community based on correlation studies (16–18). Synchrony in seasonal trends was observed by Kent, et al. (17); however, in a second year of sampling for seasonal trends in Crystal Bog and Trout Bog, these findings were not reproduced (19). Successional trends were studied in Crystal Bog and Lake Mendota in 2000-2001 and “dramatic changes” in community composition associated with drops in biodiversity are described during the summer months, while spring, winter, and fall had more stable community composition (16). Because the majority of our dataset was collected during the summer stratified period, increased summer variability may explain why we see a different community each year and a lack of seasonal trends. However, we cannot disprove the presence of seasonality in temperate freshwater lakes.

One of the biggest benefits of 16S amplicon sequecing over ARISA is the ability to classify sequences. In addition to a core of persistent taxa found in nearly every sample collected, we also identified taxa endemic to either the epilimnion or hypolimnion and to specific mixing regimes. These endemic taxa likely reflect the biogeochemical differences driven by mixing regime. Dimictic and meromictic hypolimnia, which are consistently anaerobic, harbor putative sulfur reducers not present in polymictic hypolimnia, which are more frequently oxygenated. Members of clade acI partition by mixing regime in epilimnia, though the functional traits driving this filtering effect are the subject of active study (4). Interestingly, the hypolimnion of meromictic Mary Lake contains several taxa classified into the candidate phyla radiation and a larger proportion of completely unclassified reads than other hypolimnia (20). This is consistent with the findings of other 16S and metagenomics studies of meromictic lakes, and suggests that the highly reduced and consistently anaerobic conditions in meromictic hypolimnia support taxa would make excellent study systems for research on members of the candidate phyla radiation and “microbial dark matter” (21, 22).

Perhaps the biggest implication of this study is the importance of repeated sampling of the same locations. A similar dataset spanning only a single year would not have captured the full extent of variability observed, and therefore would not have detected as many of the taxa belonging to the bog lake community. While we found no evidence for seasonal trends or repeated annual trends, it is possible that there are cycles or variables acting on scales greater than the five years covered in this dataset, or that annual differences are driven by environmental factors that do not occur every year. Biotic interactions between bacterial taxa may also contribute to the observed variability. Understanding the factors that contribute to variability in bog lake communities will lead to improved predictive modelling in freshwater systems, allowing forecasting of bloom events and guiding better management strategies. Additionally, these systems may be ideal for addressing some of the core questions in microbial ecology, such as how community assembly occurs, how interactions between taxa shape community composition, and how resource partitioning drives the lifestyles of bacterial taxa.

To answer these questions and more, we are continuing to collect and sequence samples for the North Temperate Lakes – Microbial Observatory, and we are expanding our sequencing repertoire beyond 16S sequencing. All of the 16S data we have currently generated can be found in the R package “OTUtable” which is available on CRAN for installation via the R command line, or on our GitHub page. We hope that this dataset and its future expansion will be used as a resource for researchers investigating their own questions about how bacterial communities behave on long time scales.

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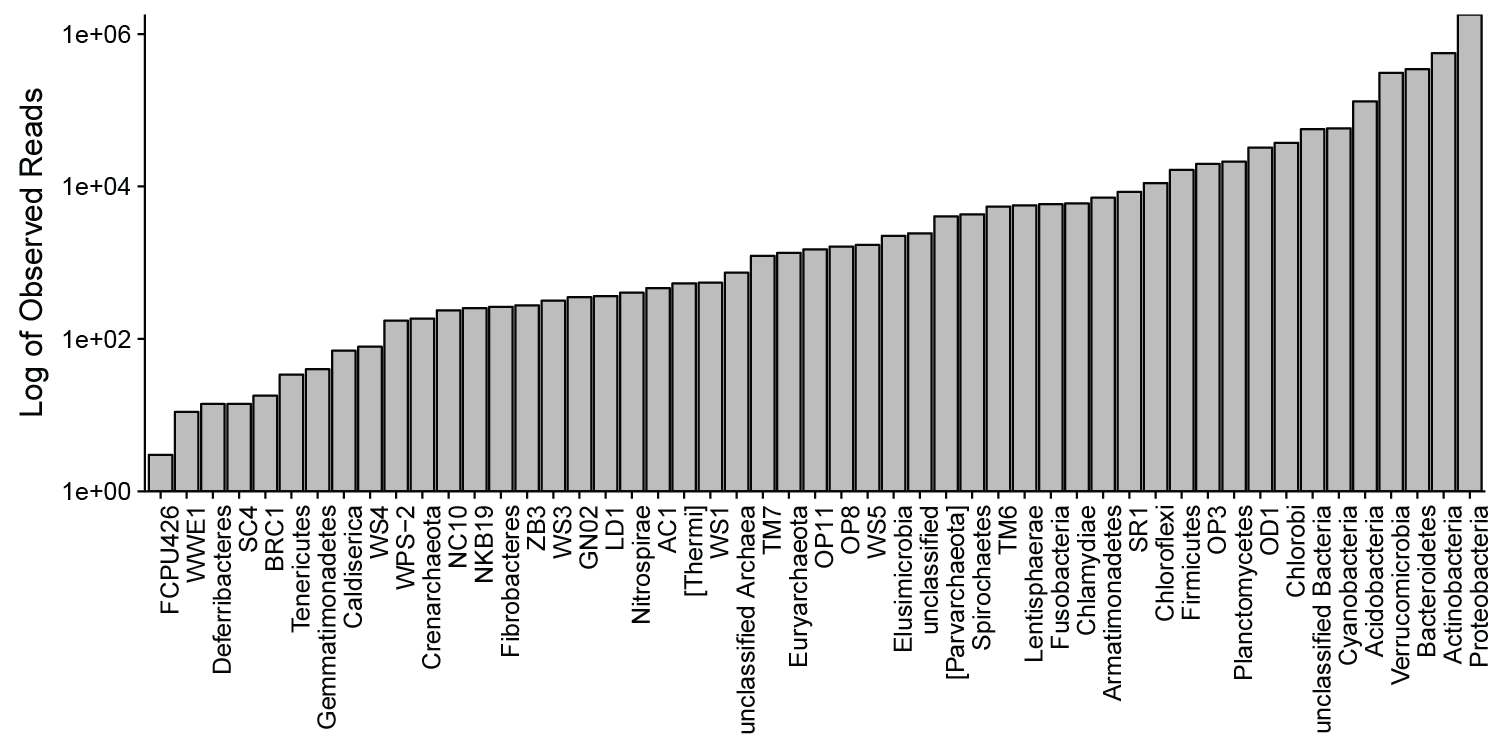
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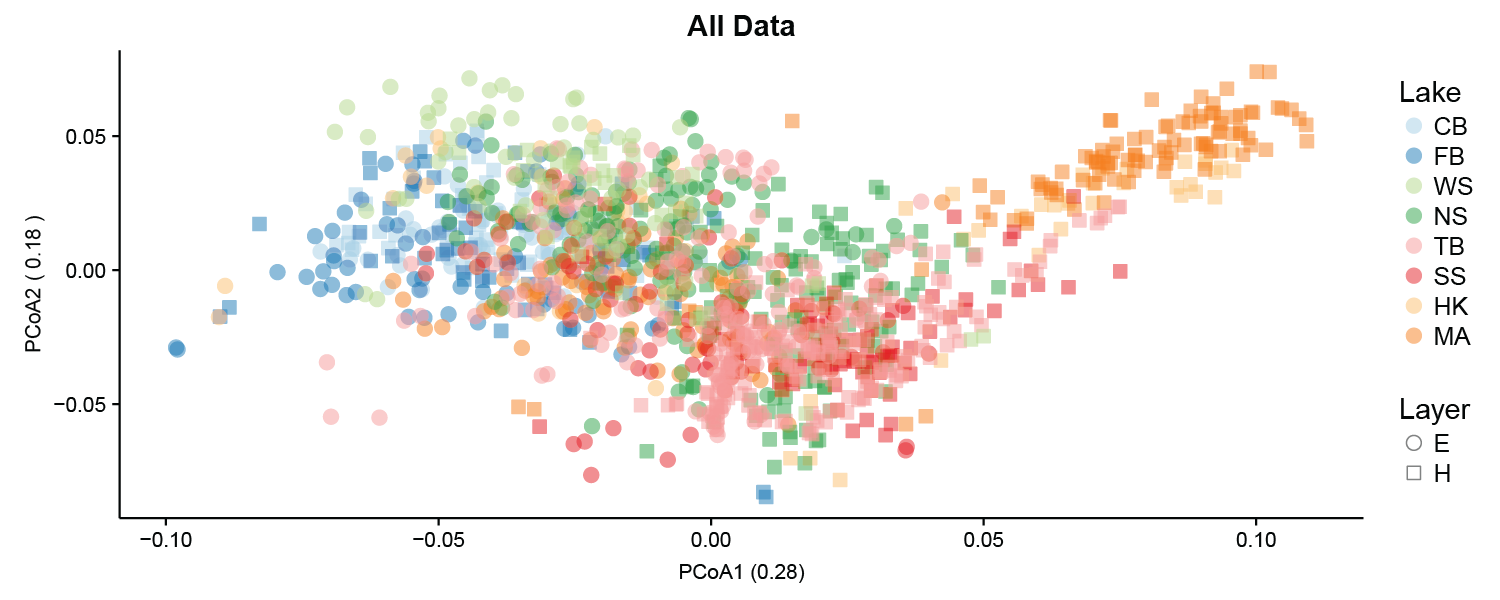
# Supplemental

**Figure S1. Phylum rank abundance in entire dataset.** When OTUs are grouped by phylum and read abundances summed over the entire dataset, Proteobacteria, Actinobacteria, and Bacteroidetes are the most abundant phyla. Unclassified Bacteria are the fifth largest group. Members of the candidate phyla radiation such as OD1 (Parcubacteria) and OP3 (Omnitrophica) are also well-represented in this dataset.

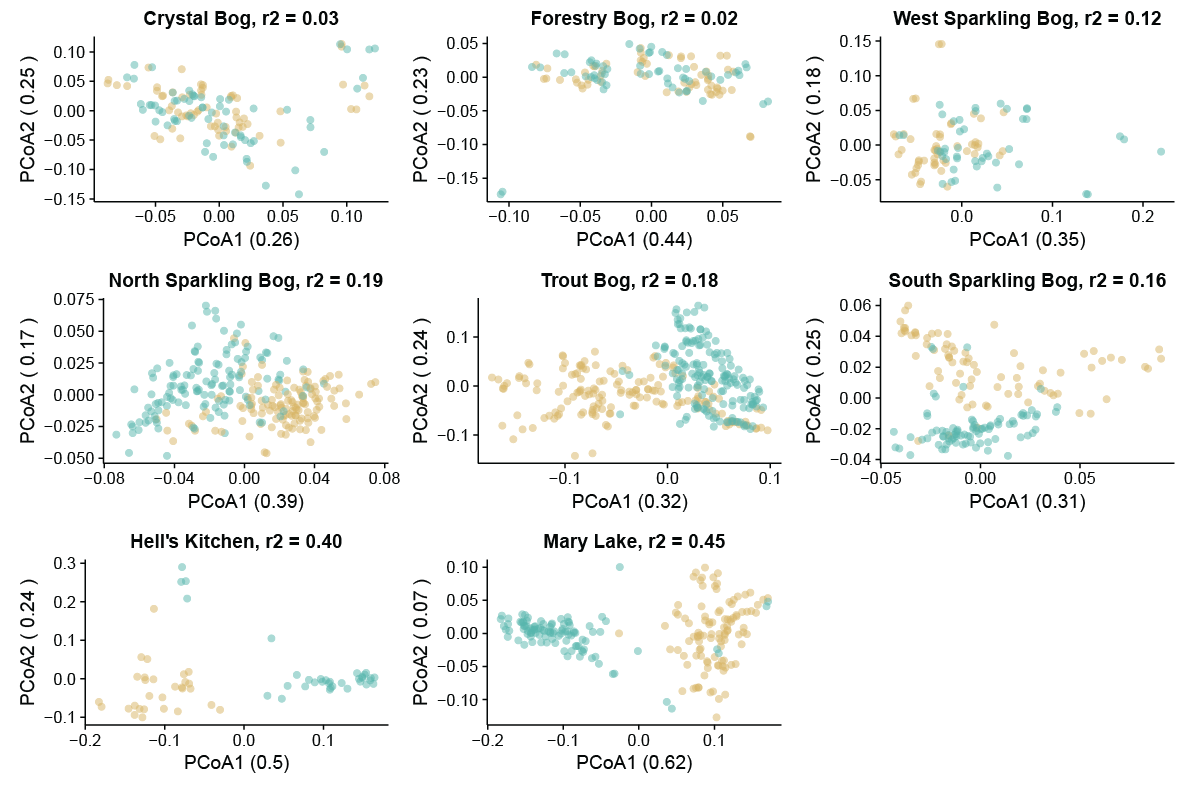
**Table S1. Means and standard deviations in the number of taxa by lake and layer.** In order to better interpret the results of Figure 1, statistics about richness are presented here.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Epilimnion  Mean | Standard Deviation | Hypolimnion  Mean | Standard deviation |
| Crystal Bog (CB) | 129 | 28 | 148 | 31 |
| Forestry Bog (FB) | 109 | 32 | 145 | 57 |
| West Sparkling Bog (WS) | 150 | 45 | 182 | 56 |
| North Sparkling Bog (NS | 143 | 33 | 178 | 40 |
| Trout Bog (TB) | 148 | 38 | 186 | 38 |
| South Sparkling Bog (SS) | 191 | 57 | 191 | 54 |
| Hell’s Kitchen (HK) | 199 | 67 | 397 | 124 |
| Mary Lake (MA) | 259 | 67 | 477 | 110 |

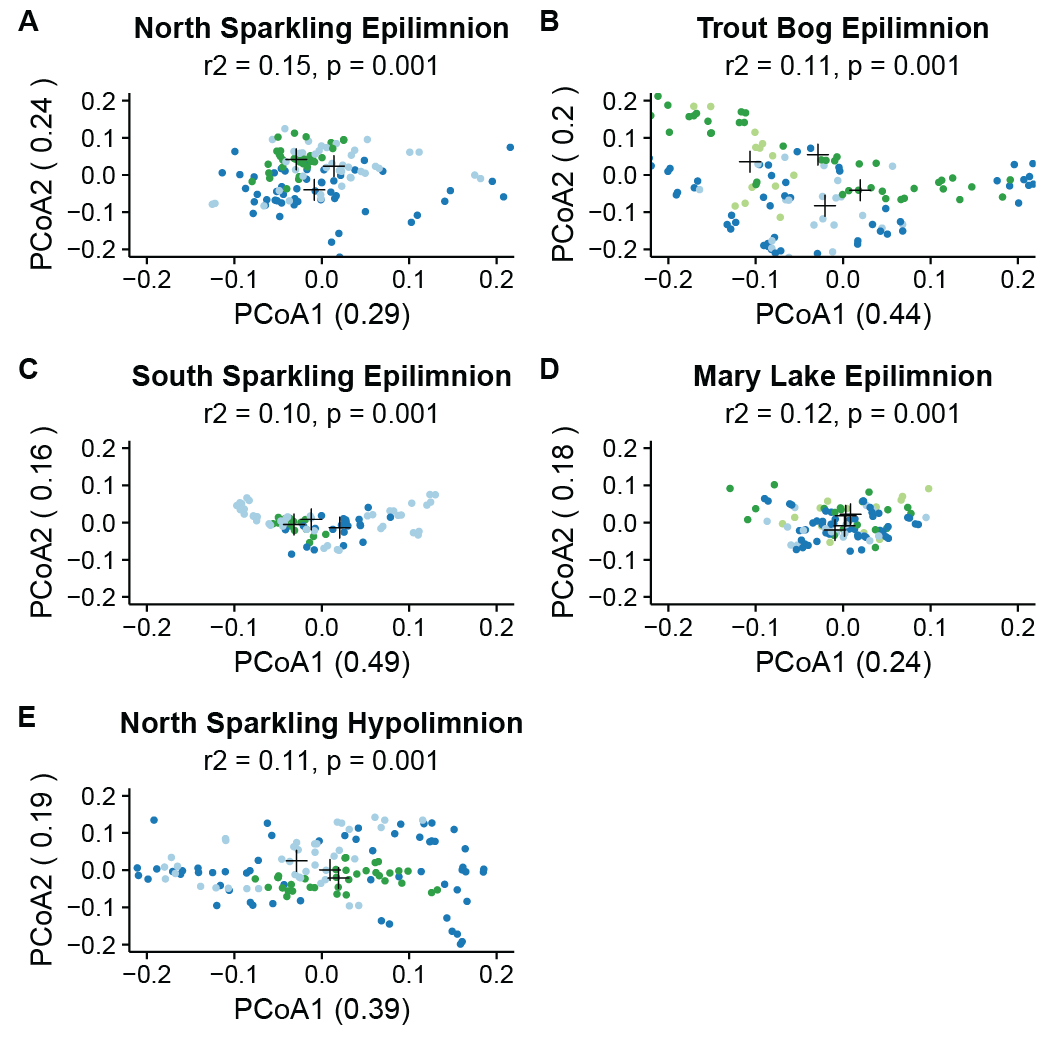
**Figure S2. PCoA of all data points with a layer designation.** As an overview of dataset, an ordination was performed on all datapoints with a layer designation using UniFrac distance and principle coordinates analysis. Samples cluster by lake, layer, and mixing regime. Polymictic epilimnia and hypolimnia samples are found together. Dimictic hypolimnia samples are distinct from dimictic epiliminia samples, but still overlap in the ordination. Meromictic hypolimnia cluster separately from the rest of the dataset.



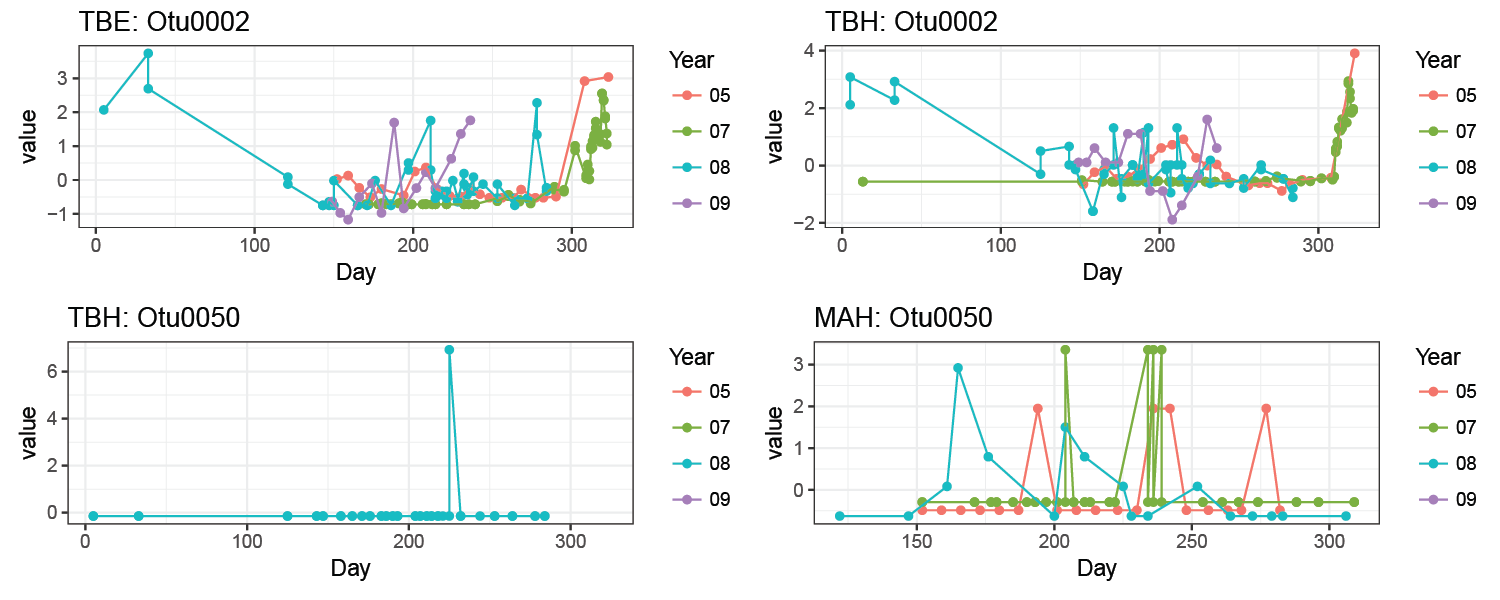
**Figure S3. Layers cluster within lakes**. All clustering by layer is significant at p < 0.005 except Forestry Bog, where p = 0.101. Given that it is polymictic, shallow, and only includes one year of sampling, this is not surprising. Clustering is especially prominent in the meromictic lakes.



**Figure S4. PCoA of extra epilimnia and hypolimnia by lake by year**. Each year in each lake has a unique community composition, regardless of layer. Plots for lakes and layers not shown in Figure 3 are presented here, using the same analysis as in the main text. Only sites with at least three years of sampling were analyzed.



**Figure S5. Annual trends in OTUs.** We could not identify repeating seasonal trends in OTU abundances. While OTUs tended to show a consistent response to mixing events, their abundance during summer stratification was variable. Example plots showing abundance trends in OTUs over multiple years in the same site are presented here, and readers curious about other OTUs and sites can run the code below for any combination of OTU and location.

****

library(OTUtable) # You will need these three packages

library(ggplot2)

library(reshape2)

data(otu\_table) # Load the OTU table

# Write function to plot multiple years at once.

annual\_trends <- function(lake, otu){

bog <- bog\_subset(lake, otu\_table)

year1 <- year\_subset("05", bog)

year2 <- year\_subset("07", bog)

year3 <- year\_subset("08", bog)

year4 <- year\_subset("09", bog)

# Since sites have different years sampled, these if statements identify which years are present

if(dim(year1)[2] > 0){

# Once years present are identified, normalize and combine into a single table

year1 <- zscore(year1)

year2 <- zscore(year2)

year3 <- zscore(year3)

year4 <- zscore(year4)

ztable <- cbind(year1, year2, year3, year4)

}else if(dim(year1)[2] == 0 & dim(year3)[2] > 0){

year2 <- zscore(year2)

year3 <- zscore(year3)

year4 <- zscore(year4)

ztable <- cbind(year2, year3, year4)

}else if(dim(year1)[2] == 0 & dim(year3)[2] == 0 & dim(year4)[2] > 0){

year2 <- zscore(year2)

year4 <- zscore(year4)

ztable <- cbind(year2, year4)

}else{

ztable <- zscore(year2)

}

# Format the final table

ztable <- melt(ztable)

ztable$Year <- substr(ztable$Var2, start = 9, stop = 10)

ztable$Day <- format(extract\_date(ztable$Var2), format = "%j")

# Save the results for plotting

plot <- ggplot(data = ztable[which(ztable$Var1 == otu), ], aes(x = Day, y = value, group = Year, color = Year)) + geom\_point() + geom\_line() + theme\_bw() + labs(title = paste(lake, otu, sep = ": "))

return(plot)

}

# Example Usage – 3 letter site code includes 1st 2 for site (see Table 1) and letter 3 for layer (E = epilimnion, H = hypolimnion. OTU designation is case sensitive, and number must contain 4 digits.

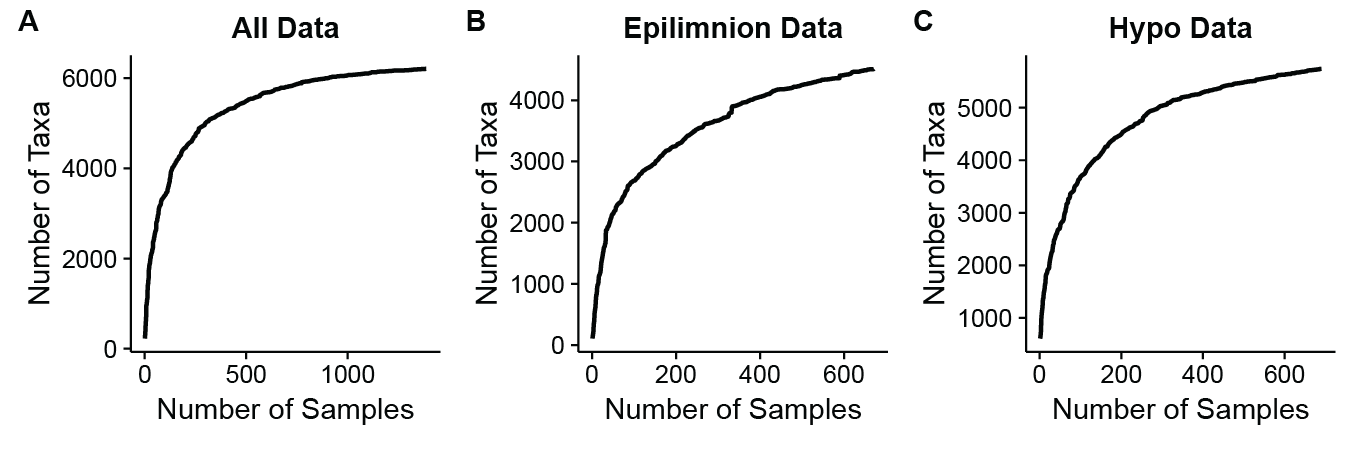
plot\_this <- annual\_trends(“TBE”, “Otu0012”)

plot\_this

# You may get warning messages about points being removed. That means the OTU was not present in those points

# If all points were removed and no plot is produeced, it was not present in that site

**Figure S6. Rarefaction curves.** Rarefaction curves generated by randomly ordering samples and counting the cumulative number of taxa show logarithmic rather than linear trends as the number of samples included increases. This suggests that we are beginning to exhaustively sample the community of bog lakes.



Supplemental document – indicator analysis results