Supplemental Figures and Tables

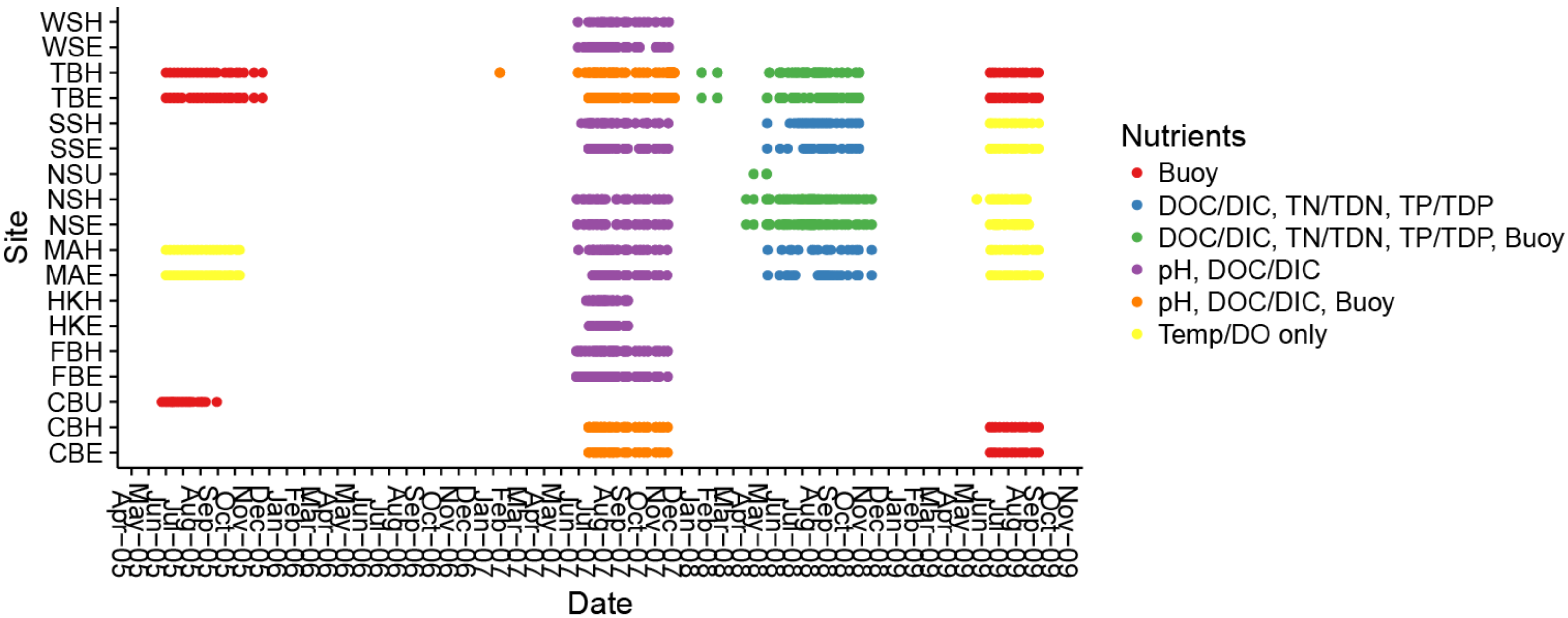


Figure S1. Sampling frequency and paired environmental data. Lakes were sampled during stratification each summer (Jun – Aug). Some sites include additional early spring samples (Apr-May) and fall samples (Sep – Nov). Not every lake was sampled in every year of sampling; every lake was sampled in 2007. Layer codes (third letter in the site designation on the y-axis) of “U” indicate that the entire water column was sampled, rather than splitting samples by epilimnion and hypolimnion. Temperature and dissolved oxygen were measured throughout the water column during the collection of every sample in our dataset. pH, DIC/DOC (dissolved inorganic and organic carbon), TN/TDN (total and dissolved nitrogen), and TP/TDP (total and dissolved phosphorus) were not measured every year in every site. Instrumented buoys, maintained by North Temperate Lakes - Long Term Ecological Research, are located on a subset of the sampling locations.

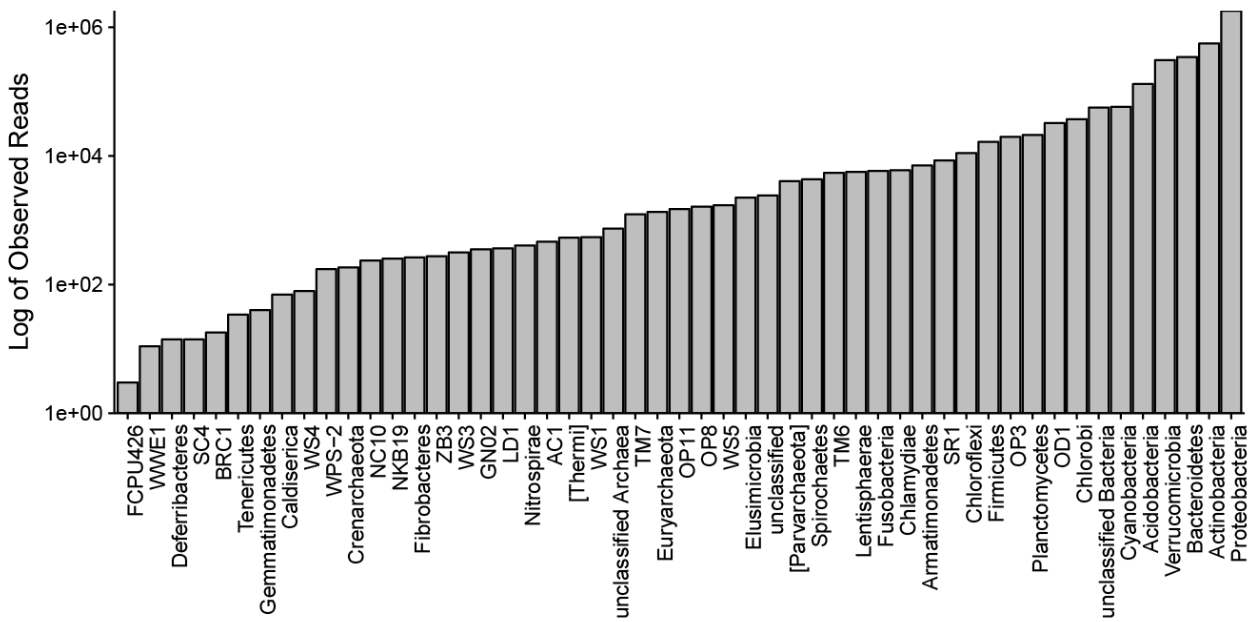
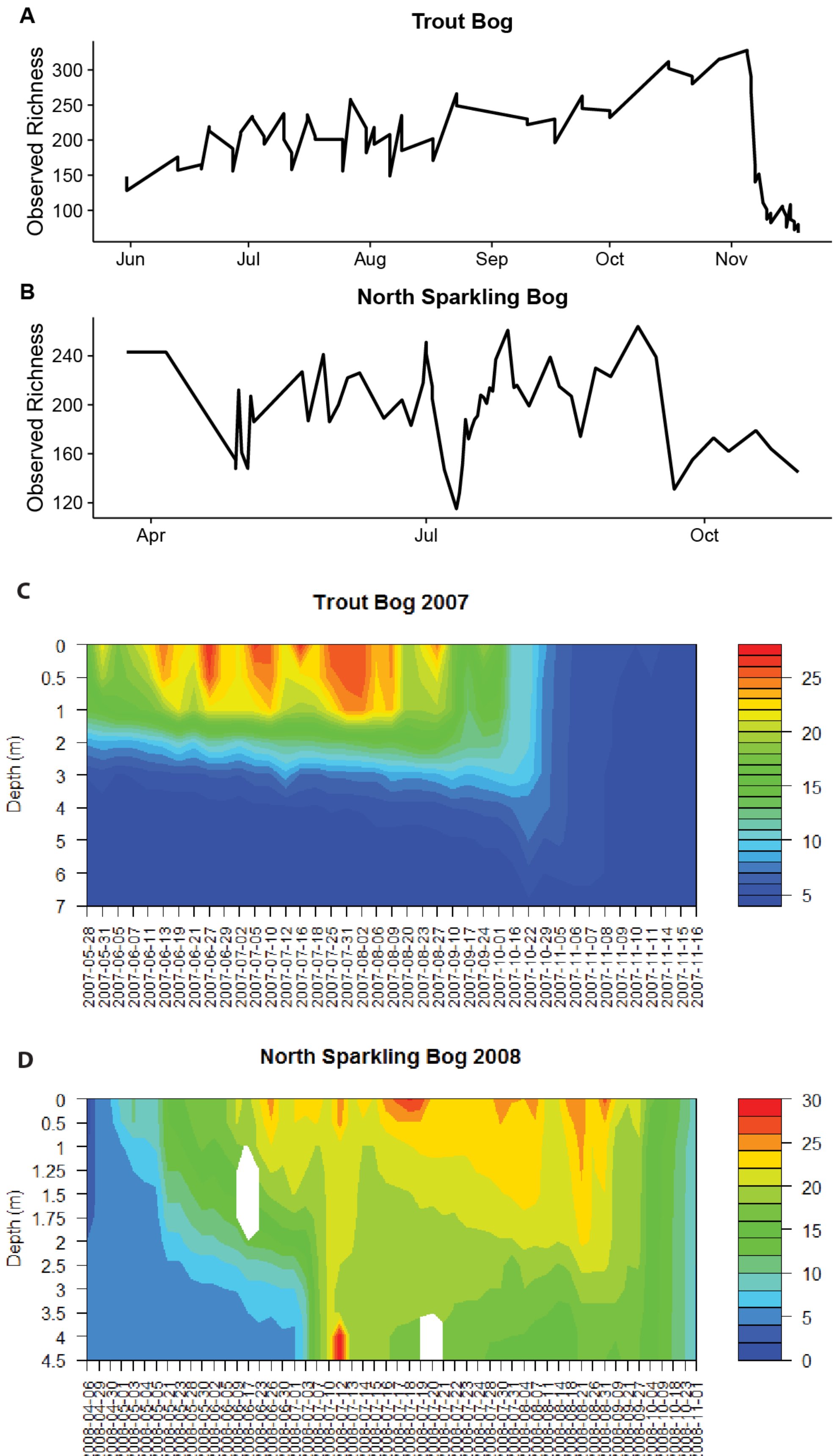


Figure S2. Phylum rank abundance in entire dataset. When OTUs are grouped by phylum and read abundances summed over the entire dataset, Proteobacteria, Actinobacteria, and Bacteroidetes are the most abundant phyla. Unclassified Bacteria are the fifth largest group. Members of the candidate phyla radiation such as OD1 (Parcubacteria) and OP3 (Omnitrophica) are also well-represented in this dataset.

Table S1. P-values from comparison of richness between sites in Figure 1. Observed richness between lakes was compared within layers using a Wilcoxon signed rank test with a Bonferroni correction for multiple pairwise comparisons.

Epilimnion			Hypolimnion		
Site 1	**Site 2**	**p-value**	**Site 1**	**Site 2**	**p-value**
FB	CB	0.01085	FB	CB	1.00000
WS	CB	0.83523	WS	CB	0.03692
NS	CB	0.12145	NS	CB	0.00001
TB	CB	0.00628	TB	CB	0.00000
SS	CB	0.00000	SS	CB	0.00000
HK	CB	0.00000	HK	CB	0.00000
MA	CB	0.00000	MA	CB	0.00000
WS	FB	0.00005	WS	FB	0.02735
NS	FB	0.00000	NS	FB	0.00002
TB	FB	0.00000	TB	FB	0.00001
SS	FB	0.00000	SS	FB	0.00000
HK	FB	0.00000	HK	FB	0.00000
MA	FB	0.00000	MA	FB	0.00000
NS	WS	1.00000	NS	WS	1.00000
TB	WS	1.00000	TB	WS	1.00000
SS	WS	0.00089	SS	WS	0.00000
HK	WS	0.01310	HK	WS	0.00000
MA	WS	0.00000	MA	WS	0.00000
TB	NS	1.00000	TB	NS	1.00000
SS	NS	0.00000	SS	NS	0.00000
HK	NS	0.00001	HK	NS	0.00000
MA	NS	0.00000	MA	NS	0.00000
SS	TB	0.00000	SS	TB	0.00000
HK	TB	0.00029	HK	TB	0.00000
MA	TB	0.00000	MA	TB	0.00000
HK	SS	1.00000	HK	SS	0.00000
MA	SS	0.00000	MA	SS	0.00000
MA	HK	0.00007	MA	HK	0.00089



A
Trout Bog
Observed Richness
100
150
200
250
300
Jun
Jul
Aug
Sep
Oct
Nov
B
North Sparkling Bog
Observed Richness
120
160
200
240
Apr
Jul
Oct
C
Trout Bog 2007
Depth (m)
0
0.5
1
2
3
4
5
6
7
25
20
15
10
5
2007-05-28
2007-05-31
2007-06-05
2007-06-07
2007-06-11
2007-06-13
2007-06-19
2007-06-21
2007-06-27
2007-06-29
2007-07-02
2007-07-05
2007-07-10
2007-07-12
2007-07-16
2007-07-18
2007-07-25
2007-07-31
2007-08-02
2007-08-06
2007-08-09
2007-08-20
2007-08-23
2007-08-27
2007-09-10
2007-09-17
2007-09-24
2007-10-01
2007-10-16
2007-10-22
2007-10-29
2007-11-05
2007-11-06
2007-11-07
2007-11-08
2007-11-09
2007-11-10
2007-11-11
2007-11-14
2007-11-15
2007-11-16
D
North Sparkling Bog 2008
Depth (m)
0
0.5
1
1.25
1.5
1.75
2
2.5
3
3.5
4
4.5
30
25
20
15
10
5
0

Figure S3. Richness over time during mixing events. In panels A and B, the black line traces the number of OTUs observed at each time point in the hypolimnion. Panels C and D show temperatures throughout the water column on each sampling date. Sharp decreases in richness are observed during both the fall mixing in Trout Bog, 2007 (A, C) and the artificial mixing in July in North Sparkling Bog, 2008 (B, D). Transient mixing dates in the fall of 2008 in North Sparkling Bog also show lower richness.

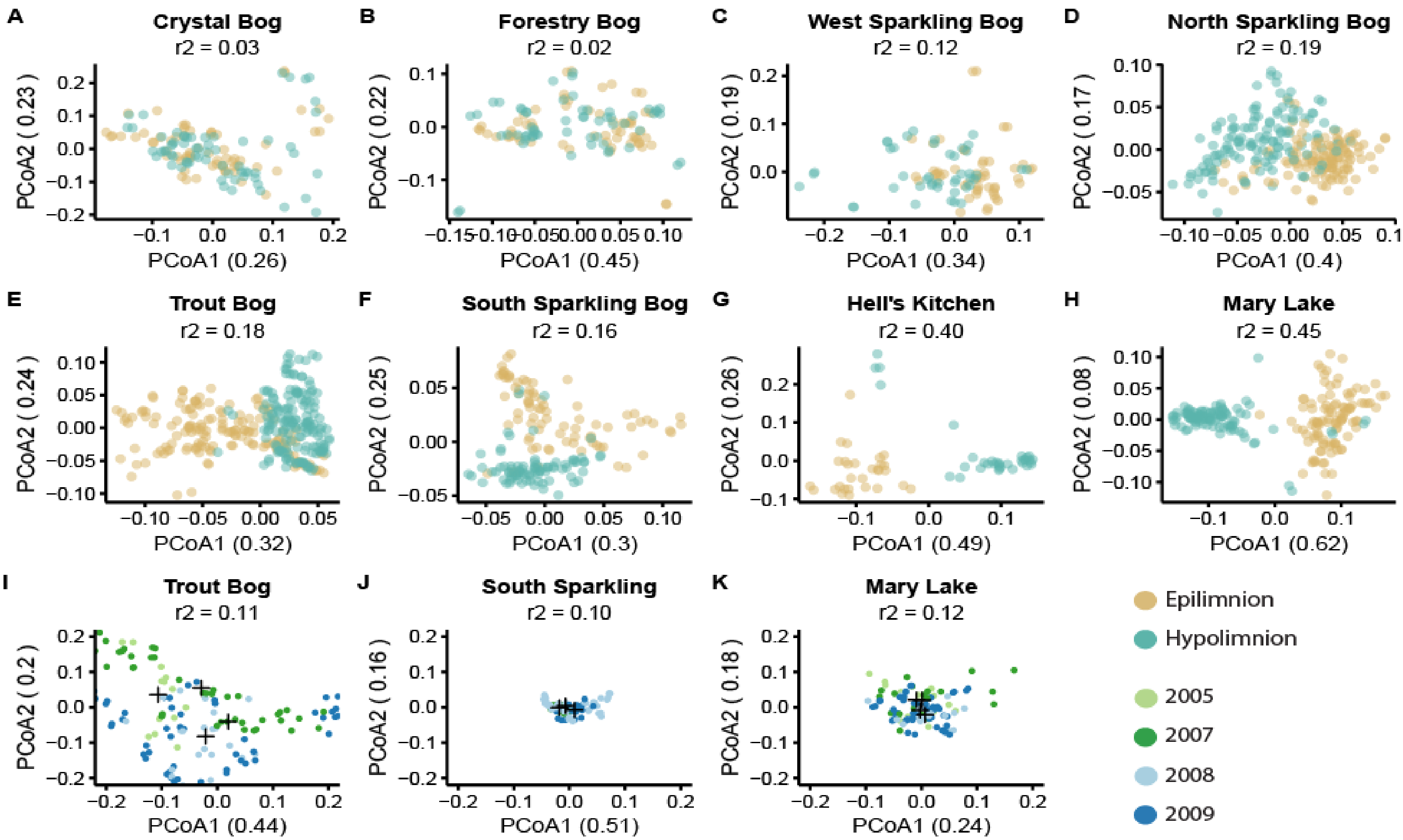


Figure S4. PCoA on subsets of the dataset. All clustering by layer (a – h) is significant at p < 0.005 except Forestry Bog, where p = 0.101. Given that it is polymictic, shallow, and only includes one year of sampling, this is not surprising. Clustering is especially prominent in the meromictic lakes. Additionally, each year in each lake has a unique community composition, regardless of layer (i-k). Plots for the epilimnia of lakes 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 and no artificial mixing events were analyzed.

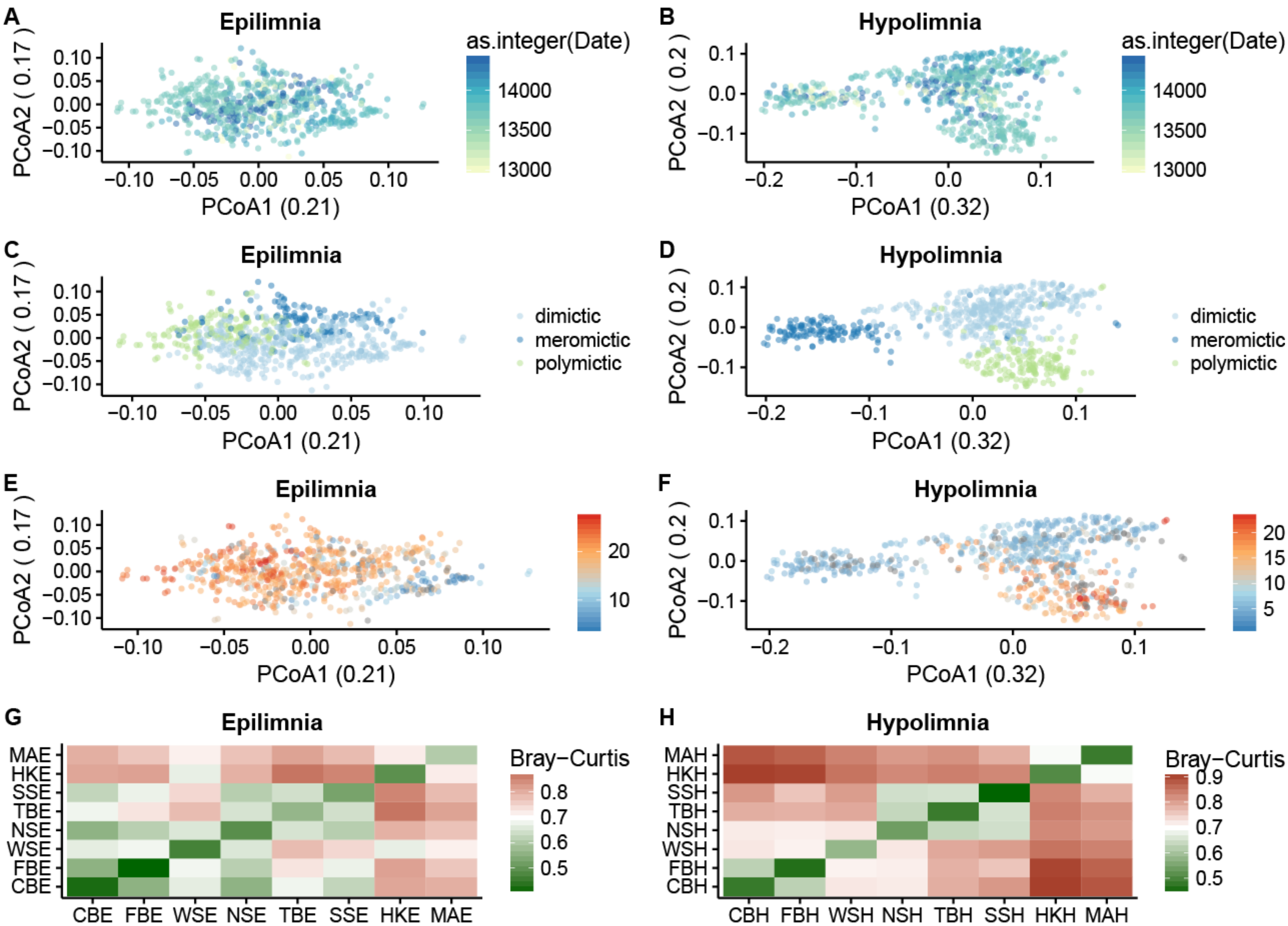


Figure S5. Alternative colorations of Figure 2. The ordination displayed in Figure 2 is presented here with different colorations representing environmental data. Panels A and B show Julian date; no association between community composition and date is observed. Panels C and D color points by mixing regime rather than lake, which was found to be a significant factor explaining community composition in both layers. Panels E and F are colored by the mean water temperature in each layer on the sampling date; results appear associated with mixing regime, especially in hypolimnia. To corroborate the conclusions drawn from ordinations in Figure 2, we investigated beta diversity between sites using a straightforward distance metric rather than principle coordinates analysis (g-h). Every sample was compared to every other sample, and the mean pairwise dissimilarity between sites is represented here. Clustering by mixing regime, particularly in hypolimnia, is still observed in this analysis.

Table S2. PERMANOVA tables. The results of PERMANOVA, implemented using adonis() from the R package "vegan," are shown here. Significant clustering by lake and mixing regime in the principle coordinates analysis in Figure 2 is supported, as is clustering by year within lakes shown in Figure 3a-c.

		Degrees Freedom	**Sums of Squares**	**Mean Squares**	**F Statistic**	**Partial r^2**	**p-value**
Epilimnion	Lakes	7	2.68	0.38	45.85	0.33	0.001***
	Residuals	663	5.53	0.01		0.67	
	Total	670	8.20			1.00	
	Regime	2	1.52	0.76	75.85	0.19	0.001***
	Residuals	668	6.68	0.01		0.81	
	Total	670	8.20			1.00	
Hypolimnion	Lakes	7	5.25	0.75	98.38	0.50	0.001***
	Residuals	682	5.20	0.01		0.50	
	Total	689	10.45			1.00	
	Regime	2	3.89	1.94	203.49	0.37	0.001***
	Residuals	687	6.56	0.01		0.63	
	Total	689	10.45			1.00	
Trout Bog	Year	3	0.30	0.10	30.25	0.36	0.001***
	Residuals	162	0.53	0.00		0.64	
	Total	165	0.83			1.00	
South Sparkling Bog	Year	2	0.11	0.06	10.57	0.20	0.001***
	Residuals	82	0.44	0.01		0.80	
	Total	84	0.55			1.00	
Mary Lake	Year	3	0.35	0.12	3.79	0.10	0.001***
	Residuals	99	3.04	0.03		0.90	
	Total	102	3.39			1.00	

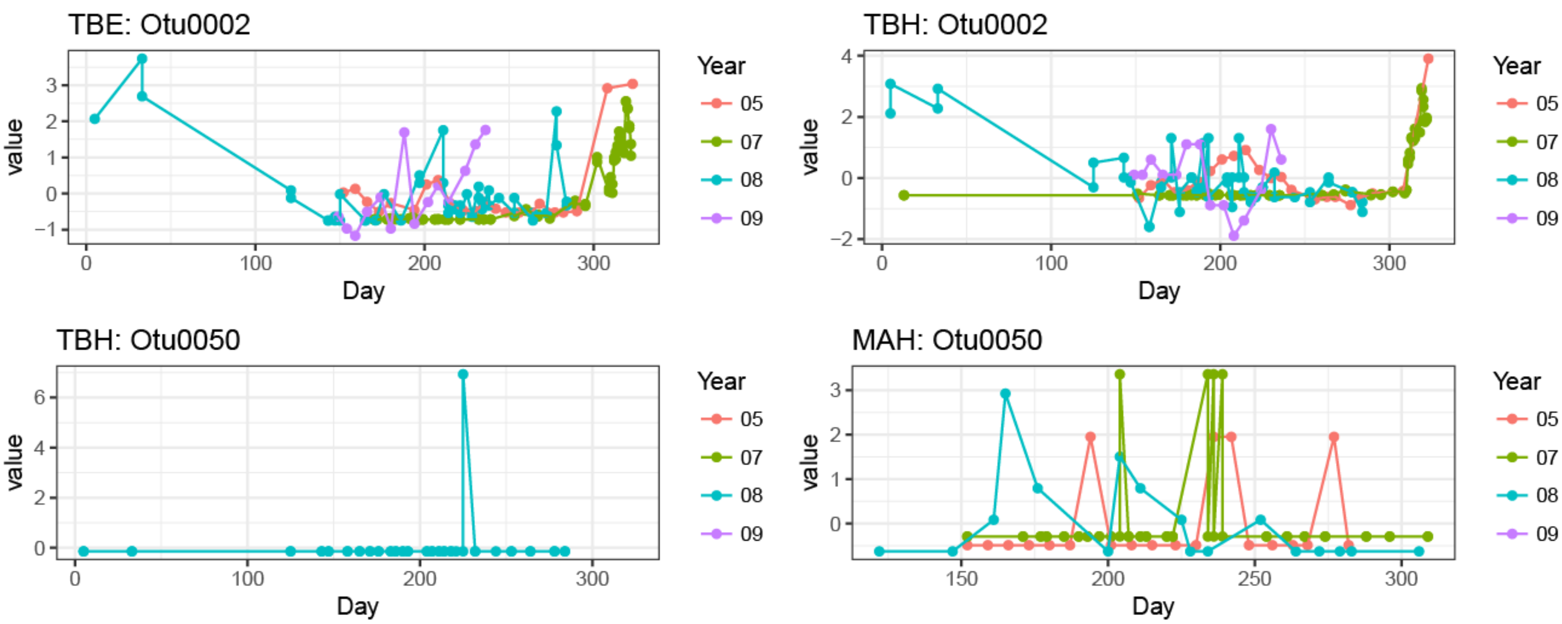


Figure S6. Annual trends in OTUs. We could not identify repeating seasonal trends in OTU abundances. While OTUs tended to show a consistent response to mixing events, their abundance during summer stratification was variable. Example plots showing abundance trends in OTUs over multiple years in the same site are presented here, and readers curious about other OTUs and sites can run the R script "annual_trends_in_OTUs.R" at https://github.com/McMahonLab/North_Temperate_Lakes-Microbial_Observatory for any combination of OTU and location.

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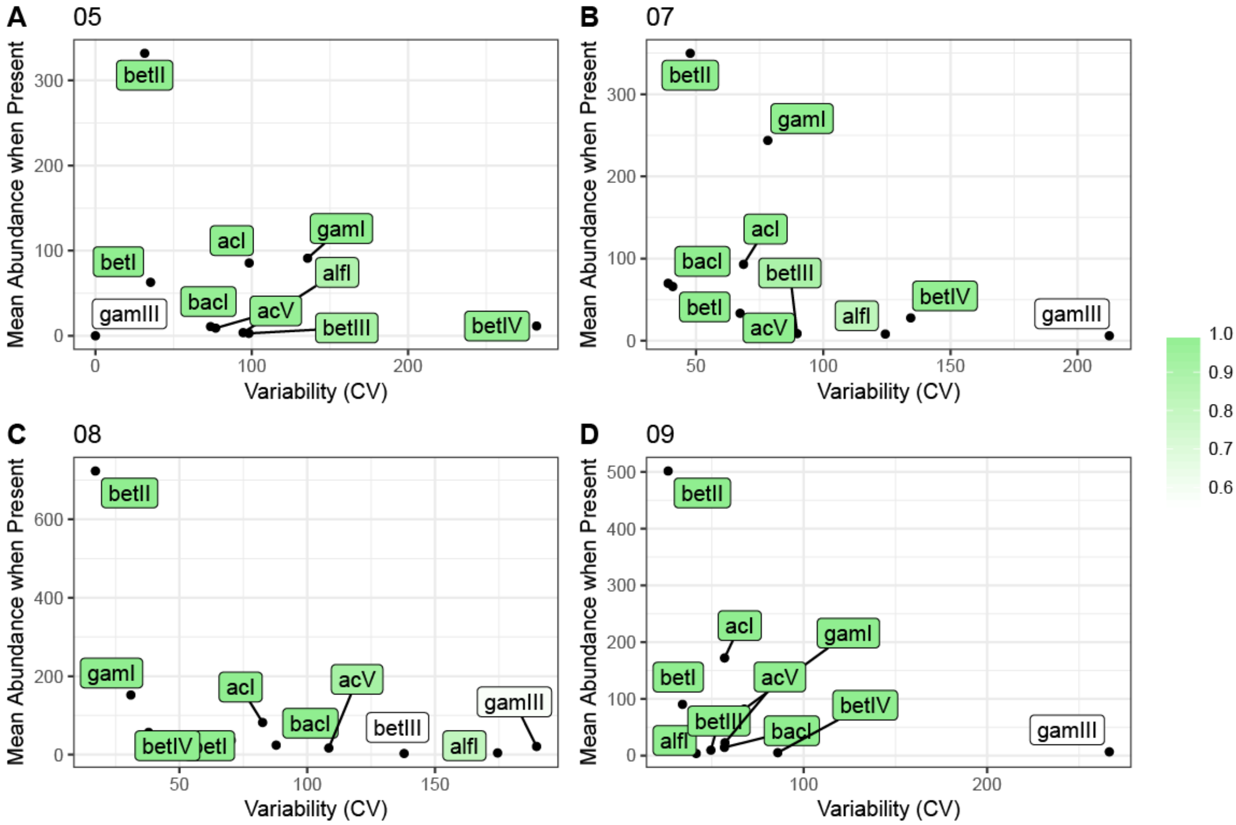

Figure S7. Lineage traits by year. Figure 5 demonstrates that lineages show consistent traits in different lakes; this plot shows that those traits are relatively consistent between years in the Trout Bog hypolimnion as well.