SUPPLEMENTAL MATERIAL FOR

Carbon and nitrogen recycling during cyanoHABs in dreissenid invaded and non-invaded US midwestern lakes and reservoirs

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DISCUSSION

TOTAL CARBON

The overall C concentration at GLSM in the surface sediments is 3.4 mol C/kg for organic carbon and 1.8 mol C/kg for carbonate carbon, and at Buckeye Lake 3.5 mol C/kg for organic carbon and 1.7 mol C/kg for carbonate carbon. These give totals of 5.3 mol C/kg buried in the surface sediments at GLSM and 5.1 mol C/kg at Buckeye Lake, or about half that estimated for eutrophic lakes in Minnesota (Dean, 1999). These data suggest shallow eutrophic lakes or reservoirs impacted by blooms are efficient at removing organic C from the water column, and thus potentially efficient at sequestering C.

The overall surface sediment C concentration at Brookville Lake and Sandusky Bay were the lowest values measured in this study, at 4.13 mol C/kg and 4.37 mol C/kg, respectively. However, at Brookville Lake most of the C was present as carbonate (3.62 mol C/kg) while at the Sandusky Bay site most of the C was present as organic carbon (2.68 mol C/kg). The Sandusky Bay site was from a protected mud flat (with no invasive mussel shells or shell fragments present in the sediment sample) where anoxia near the water-sediment interface coupled to the predominantly silt and clay sediments may inhibit colonization by invasive mussels locally, while the greater bay and Lake Erie area (including the rocky substrates of the nearby jetty a few meters away) are more densely populated by mussels. The Brookville Lake site had sediments that ranged in size from sand to gravel, and included abundant invasive mussel shells and shell fragments, indicating an active invasive mussel colony present or very close to the sampling location. Regardless of this local heterogeneity of mussel population density, both sites exhibited the lowest C concentration values for the surface sediments, suggesting that cyanoHABs coupled to the presence of invasive mussel species may in fact reduce carbon deposition and thus C sequestration potential.

CONCEPTUAL MODEL

Our data suggest the presence or absence of invasive mussel species is not correlative to the composition of cyanobacteria in cyanoHABs. However, we acknowledge that our single time point samples do not capture temporal or seasonal variability in bloom community structure. Regardless, we examined sites with and without invasive mussel species that experience cyanoHABs annually to begin to constrain how the presence of invasive mussels may affect carbon sequestration in eutrophic systems. We constructed a conceptual model for the movement and sequestration of carbon in the different types of lakes and reservoirs sampled in this study (Fig. 9 in the main text).

Lakes and reservoirs that have experienced eutrophication that drives cyanoHABs such as Buckeye Lake and GLSM have potential for efficient sequestration of both C_{org} and C_{carb}. However, the C_{org} sediment concentrations at those two lakes (4.14 to 4.17 %) indicate an approximate three-fold reduction in C_{org} burial compared to an average of surface sediments at 20 lakes across Minnesota (Dean, 1999). Furthermore, the C_{carb} sediment concentrations (1.99 % at Buckeye Lake and 2.17 % at GLSM) indicate less carbonate burial compared to an average of 3.13 % C as $CaCO_3$ (Dean, 1999). From summing the averages of Buckeye Lake and GLSM, the carbon burial in the surface sediments of cyanoHAB-impacted lakes and reservoirs is 6.2 % (5.19 mol C/ kg dry sediment), or about 54 % reduction in C burial compared to that of Minnesota lakes (average of 11.21 mol C/kg dry sediment; Dean, 1999), suggesting the Ohio lakes are less effective at burying C. Loss of C_{org} as CH_4 due to breakdown of C_{org} by methanogens may also play an important role in impacting the carbon sequestration potential of Ohio lakes, which warrants further study.

Lakes and reservoirs with cyanoHABs which have also been colonized by invasive mussel species (such as Brookville Lake and Sandusky Bay, Lake Erie) appeared to exhibit a reduced capacity for carbon sequestration compared to the cyanoHAB-only sites (Fig. 9 in the main text). Reduced burial of organic carbon is likely linked directly to consumption and respiration/metabolic breakdown of organic matter from the water column by mussels. Burial of inorganic carbon may be influenced by several factors. First, reduced cyanobacterial biomass may be related to decreased rates of photosynthetically driven calcite production due to decreased nucleation sites (fewer cells in the water column). Second, increased uptake of calcium by mussels for shell formation may lead to less calcium available for calcite precipitation. And, finally, increased growth of mussels may lead to increased production of CO_2 in the water column (due to mussel metabolism) generating carbonic acid which will react with calcite crystals in the water column to dissolve it. Local heterogeneity of mussel population density can lead to a range of values for C_{org} and C_{carb}, with higher C_{org} and lower C_{carb} with lower local mussel population density. But total C values were similar for both the lower local mussel density site (Sandusky Bay, 5.24 %) and higher local mussel density site (Brookville Lake, 4.96 %), giving an average of 5.1 % C, or an average of 4.25 mol C/kg dry sediment. This represents a nearly 20 % reduction in C burial compared to cyanoHAB-only sites, or nearly a 62 % reduction in C burial compared to eutrophic lakes in Minnesota (Dean, 1999).

One caveat for Brookville Lake's carbon burial potential involves mussel shells as a means for C_{carb} sequestration. For this study, while we did not take into account carbon sequestered as carbonate precipitated as shells by mussels, invasive mussel species shells collected from Lake Erie (n = 27) showed that a mature Zebra mussel shell (two produced per individual mussel) had an average long axis of 23.4 mm (± 3.35 mm) and weighed an average of 0.378 g (± 0.149 g), containing a total of 3.78 mmol C as $CaCO_3$. Using mussel shells to compensate for the surface sediments of the Brookville Lake site (total of 4.13 mol C/kg dry sediment) to match the average C burial of surface sediments in Minnesota lakes (11.21 mol C/kg dry sediment), each kilogram of dry surface sediment deposited at Brookville Lake site would also require the production of 1875 mussel shells. Assuming a loose packing volume of 1.28 cm^3 per mussel shell (based on the shells measured for this study), the volume of 2856 mussel shells would be ~ 2,400 cm^3. Assuming a sediment density of ~ 1 g/cm^3 for Brookville Lake surface sediments, this would mean for every 1000 cm^3 of sediment, you would need nearly a 2.5 fold equivalent volume of mussel shell deposition. In other words, a 10 cm x 10 cm surface would have 10 cm deep sediment layer, and an additional 24 cm of mussel shells. This level of mussel shell accumulation could be further complicated by low concentrations of calcium in fresh water systems as well as remineralization of organic carbon producing CO_2, generating carbonic acid ($CO_2 + H_2O \Rightarrow H_2CO_3$), and potentially driving carbonate dissolution ($H_2CO_3 + CaCO_3 \Rightarrow Ca^{2+} + 2\ HCO_3^-$) in anoxic sediments.

REFERENCES

Dean W. E. and Gorham E. (1998) Magnitude and significance of carbon burial in lakes, reservoirs, and peatlands. *Geology* 26: 535–538.

Table S1. Physical characteristics of lakes sampled in this study.

Lake/Bay	Surface area (km^2)	Max depth (m)	mean depth (m)
Grand Lake St. Marys	58.3	3.0	1.8
Buckeye Lake	12.9	4.3	1.8
Kiser Lake	1.6	3.7	1.9
Brookville Lake	21.3	36.0	4.9
Sandusky Bay, Lake Erie	77.0	3.4	1.8

Table S2. Accession numbers and file names for the 16S and 18S rRNA amplicon libraries included in the present study.

	BioSample Accession	
	16S rRNA	18S rRNA
Kaiser Lake - 0m	SAMN07125357	SAMN07125357
Kaiser Lake sediments	SAMN07125358	SAMN07125358
GLSM - 0m	SAMN07125359	SAMN07125359
GLSM - 2m	SAMN07125360	SAMN07125361
GLSM sediments	SAMN07125361	SAMN07125360
Maumee River (upper) - 0m	SAMN07125362	SAMN07125362
Maumee River sediments (upper)	SAMN07125363	SAMN07125363
Auglaize River - 0m	SAMN07125364	SAMN07125364
Maumee River (lower)	SAMN07125365	SAMN07125365
Lake Erie - Bay View Boat Launch	SAMN07125366	SAMN07125366
Lake Erie - Sandusky Bay - 0m	SAMN07125367	SAMN07125369
Lake Erie - Sandusky Bay - 1.3m	SAMN07125368	SAMN07125367
Lake Erie - Sandusky Bay sediments	SAMN07125369	SAMN07125368
Lake Erie - East Harbor State Park - 0m	SAMN07125370	SAMN07125370
Sandusky River - 0m	SAMN07125371	SAMN07125371
Buckeye Lake - 0m	SAMN07125372	SAMN07125372
Buckeye Lake - 0.4m	SAMN07125373	SAMN07125373
Buckeye Lake Sediments	SAMN07125374	SAMN07125374
Brookville Reservoir - 0m	SAMN07125380	SAMN07125379
Brookville Reservoir - 3.5m	SAMN07125381	SAMN07125380
Brookville Reservoir - 6.5m	SAMN07125382	SAMN07125381
Brookville Reservoir sediments	SAMN07125383	SAMN07125382
Ohio River - 0m	SAMN07125384	SAMN07125383
Licking River - 0m	SAMN07125385	SAMN07125384

Figure S1. qPCR of SSU rRNA genes from reservoirs, rivers, and lakes in Ohio, Kentucky and Indiana. Results are presented as the mean of triplicate qPCR assays and error bars represent the standard deviation of replicates. Copy number is normalized to per gram biomass C (Table 1). Sample collection depths are indicated when more than just the surface was collected. Seds, sediments collected at the water sediment interface.

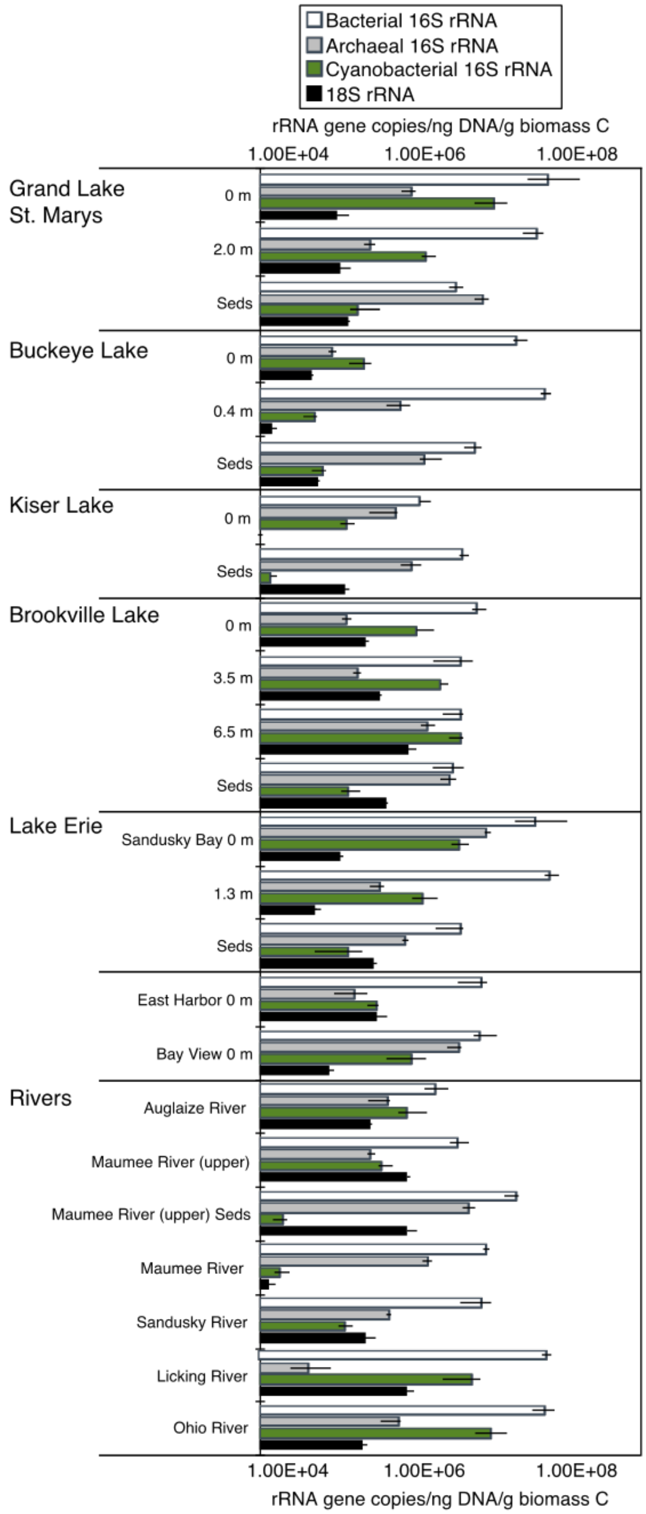

Figure S2. Nonmetric multidimensional scaling (NMDS) plot showing dissimilarities of 16S (A) and18S rRNA (B) gene sequences among the samples with Bray–Curtis distances.

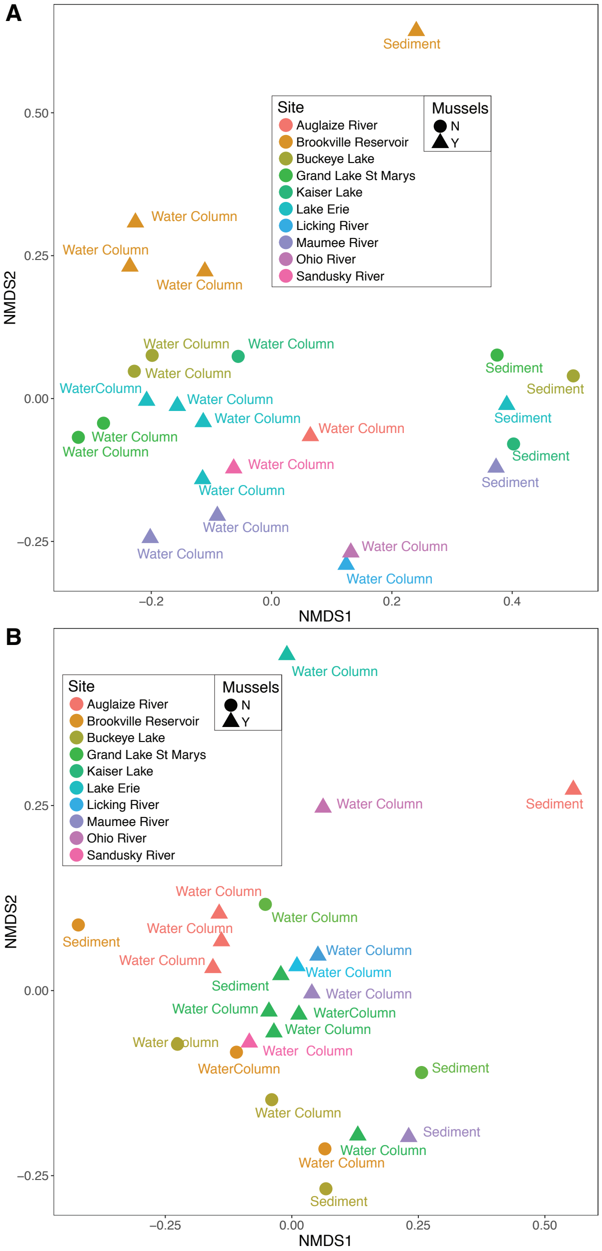

Figure S3. Composition of 16S rRNA gene sequences affiliated with Archaea recovered from sediment and planktonic biomass in reservoirs, rivers, and lakes in Ohio, Kentucky and Indiana. Representative OTUs for each library were binned at the Order level. Sample collection depths are indicated when more than just the surface was collected. Seds, sediments collected at the water sediment interface; BVBL, Bay View Boat Launch; EHSP, East Harbor State Park.

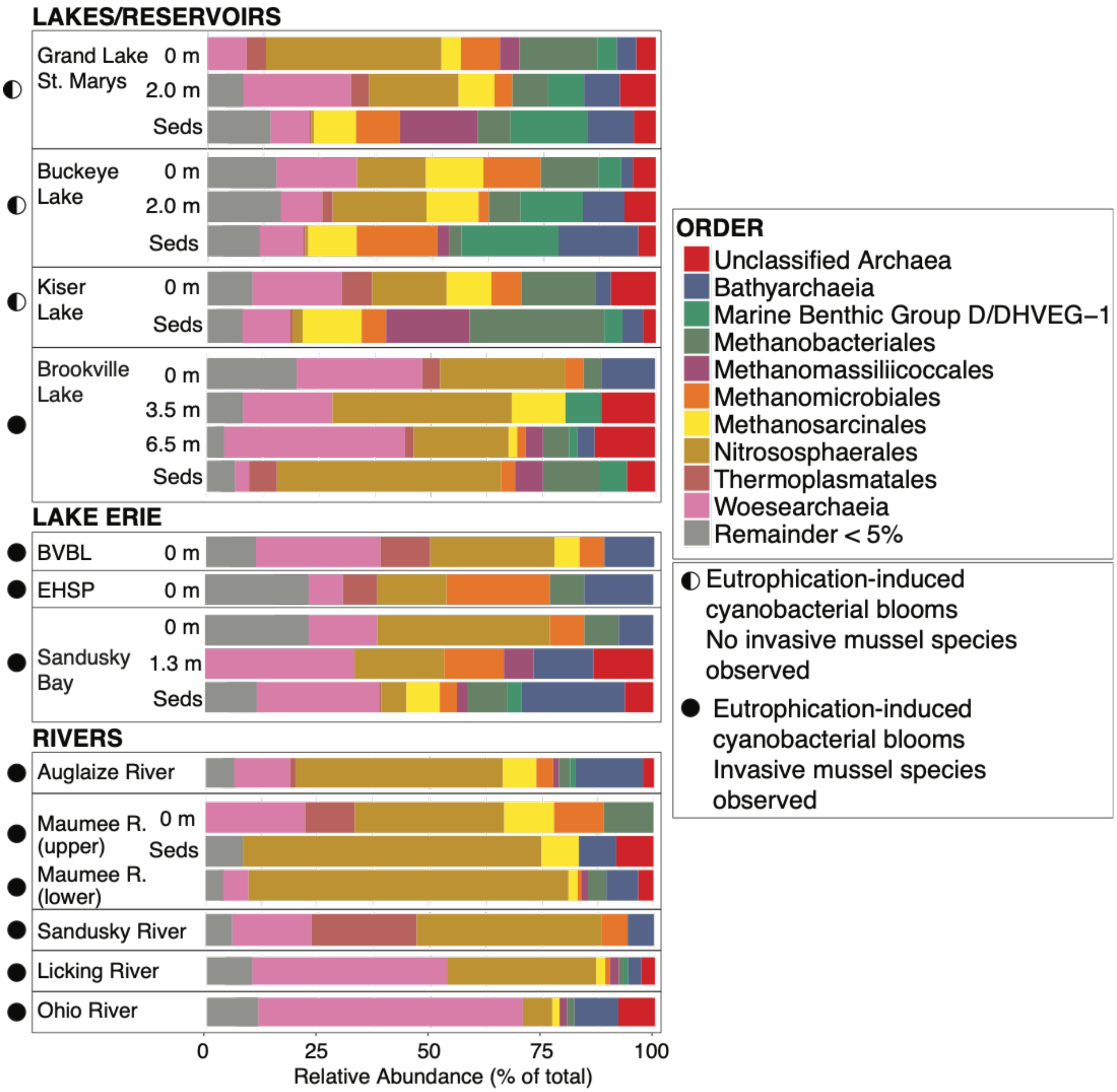

Figure S4. Composition of 18S rRNA gene sequences recovered from sediment and planktonic biomass in reservoirs, rivers, and lakes in Ohio, Kentucky and Indiana. Representative OTUs for each library were binned at the Class level. Sample collection depths are indicated when more than just the surface was collected. Seds, sediments collected at the water sediment interface; BVBL, Bay View Boat Launch; EHSP, East Harbor State Park.

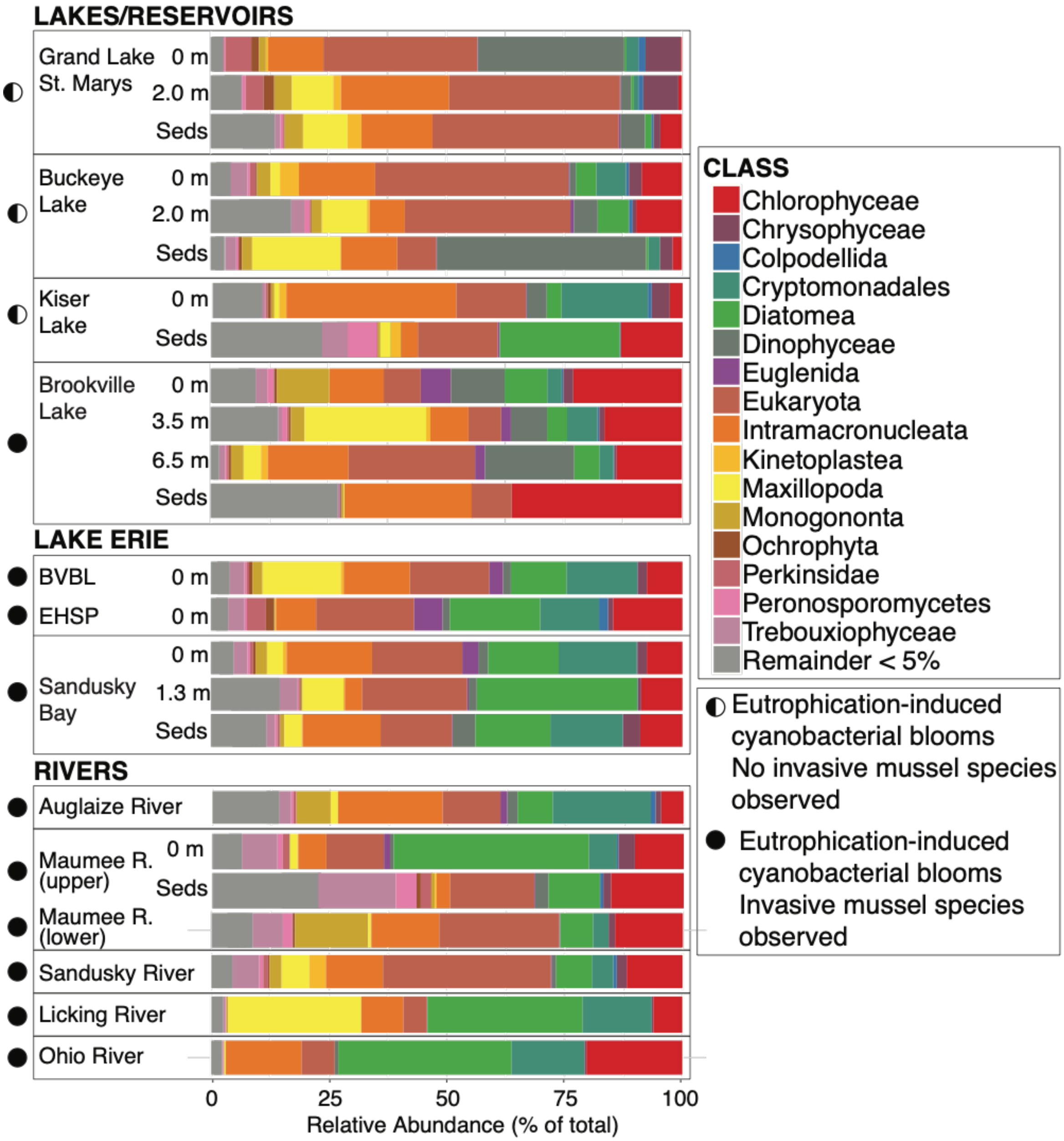

Figure S5. Alpha diversity metrics of 16S rRNA gene sequences recovered from reservoirs, rivers, and lakes in Ohio, Kentucky and Indiana. Diversity metrics are plotted by by sample type (water column or sediment) in (A) and by the presence (+) or absence (-) of mussels in (B).

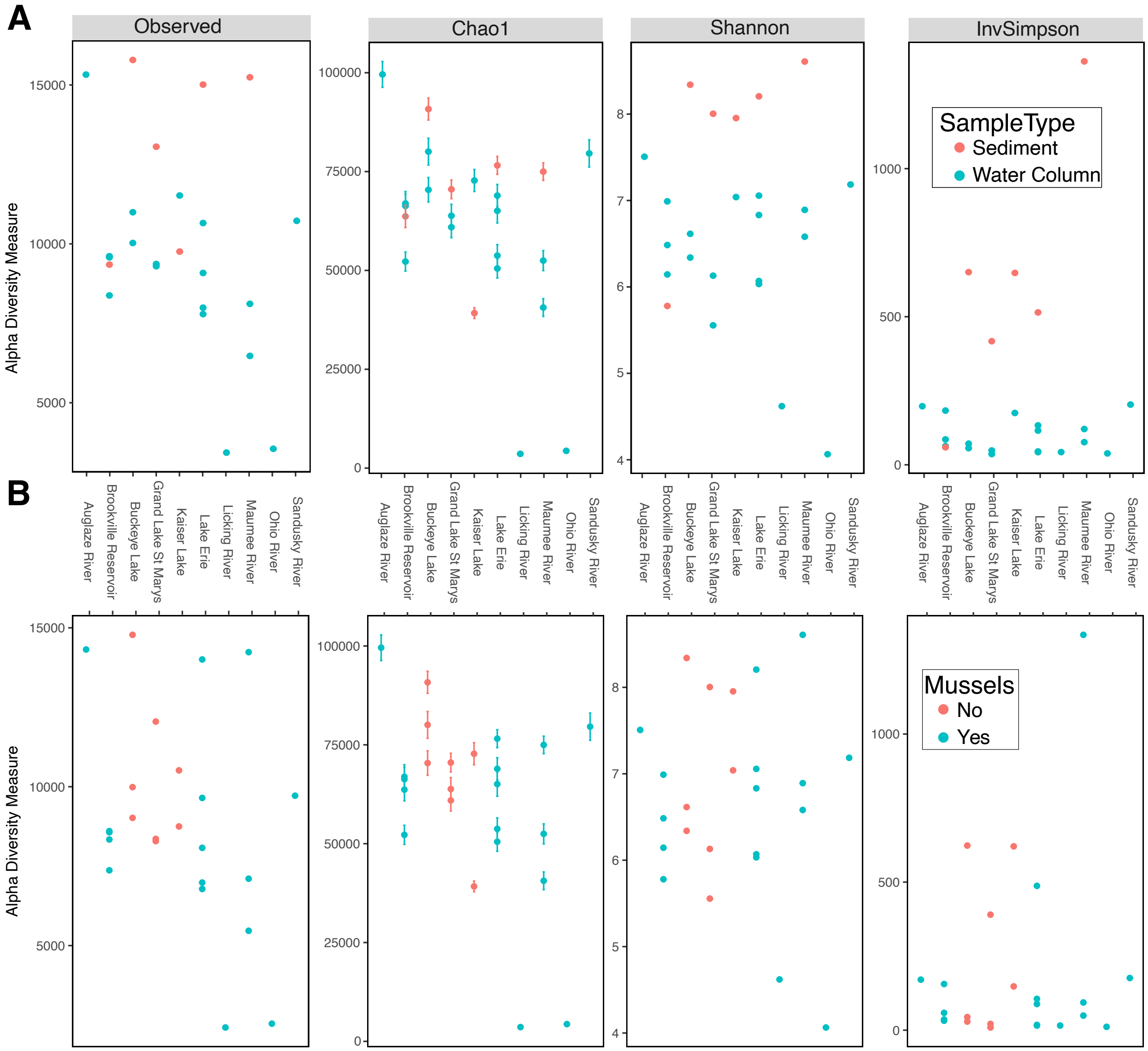

Figure S6. Alpha diversity metrics of 18S rRNA gene sequences recovered from reservoirs, rivers, and lakes in Ohio, Kentucky and Indiana. Diversity metrics are plotted by by sample type (water column or sediment) in (A) and by the presence (+) or absence (-) of mussels in (B).