Assessment of the Effectiveness of Muck-Digesting Bacterial Pellets

Final Report

Prepared for:
Dale E. Twing, Newaygo County Drain Commissioner
Erick Elgin, Newago County Extension Office, MSU Extension

Prepared by:
Emily Kindervater, Adjunct Research Assistant
Maggie Oudsema, Research Assistant
Michael Hassett, Scientific Technician
Charlyn G. Partridge, Ph.D., Professor
*Alan D. Steinman, Ph.D., Director and Professor

Annis Water Resources Institute, Grand Valley State University,
740 W. Shoreline Drive, Muskegon, MI 49441

*Contact information: steinmaa@gvsu.edu

April 2021

Executive Summary

A study was conducted using sediment from three lakes in Newaygo County (MI) to assess the ability of Mukk Buster pellets to "decrease levels of organic sediment in lakes and ponds while reducing odors and improving water clarity", as claimed on their product label. Controlled laboratory experiments were conducted using both core tubes and larger bins. Sediment from the 3 lakes were placed in enclosed, cylindrical tubes and incubated in growth chambers for 8 weeks under a 3 x 2 factorial design, with the 3 treatments being: 1) temperature (ambient or ambient plus 3ºC); 2) oxygen level (dissolved oxygen present or absent); and pellets (present or absent). Sediment from the 3 lakes were also placed in non-enclosed bins to provide a larger surface area and mass of organic matter, with or without pellets.

Changes in organic matter, dissolved organic carbon, bacterial community composition, and water quality were assessed at various times throughout the experiments. Regardless of treatment factors or sediment volume, there were no statistically significant differences in changes of organic matter between treatments with pellets and those without pellets. While the absence of a pellet effect was consistent among lakes and treatments, the change in organic matter did differ among lakes, with a slight to no increase in Hess Lake, a significant increase in Brooks Lake, and a significant decline in Pickerel Lake. We have no mechanistic explanation for the increase in organic matter in Brooks Lake, but because it occurred in both the tubes and bins, we believe the increase to be real and not due to human error. The relative number of bacterial genera was consistent across lakes, but some bacteria dominated more in specific lakes. Overall, bacteria community composition had very little explanatory power for our results. The overlying water in the pellet treatments had significantly greater specific conductance and chloride concentrations, but not pH, than in the control treatments. We attribute these increases to the contents of the pellets themselves, given that they were introduced at a much greater dosage than recommended due to the small size of our containers. It is possible the high chloride concentrations could have significant negative impacts to biota in lakes.

We identify several caveats in our study and recommend as a next step that pellet effectiveness be tested in natural lakes, using cylindrical tubes to isolate areas of sediment and water column, with control and treatment replicates. Based on these results, we conclude that these pellets are not an effective treatment to reduce sediment organic matter.

Introduction

A concern of many lakefront homeowners is the development of organic matter, also referred to commonly as “muck”, in the littoral or benthic zones of their lake. Technically, muck soils are defined by the USDA NRCS as sapric organic soils (i.e., decomposed to point where plant material is unidentifiable) that are saturated more than 30 cumulative days in normal years or are artificially drained (NRCS 2012). Concern regarding muck is typically associated with degraded aesthetics and lake in-filling, which results in impaired recreational use for those who live near, or want to use, the water body. Lake management consultants can offer bacterial pellets that “digest muck” as a management option, and which putatively reduce organic matter, along with other options that are more invasive and expensive, such as dredging.

However, muck-reducing products have not been validated in the peer-reviewed scientific literature. A non-peer reviewed study assessed the effectiveness of a combined microbial addition and aeration system in Lake Apopka, FL (Slagle and Allen 2016). The combination system had little effect on muck removal at one site and limited evidence for some muck removal at another site. At both sites, overlying water chemistry in treated areas was not significantly different than adjacent control areas. Limited sampling and environmental variation preclude definitive conclusions regarding the muck reduction treatment.

A mechanistic explanation by which pelleted bacteria consume and reduce organic matter is not available publicly, nor is a detailed composition of the pellets. The pellets used in the current study, Mukk Busster (Cygnet Enterprises, Inc., manufactured by AirMax), are stated to release bacteria as well as the enzymes lipase, protease, amylase, and cellulase to break down excess organic matter. In this study, we assessed the effectiveness of these pellets in reducing organic matter through small scale environmentally-controlled experiments in tubes and larger scale experiments in storage bins, all within a lab setting. The advantages and limitations to our experimental approach are addressed in the Discussion section of this report.

In addition to analyzing the effectiveness of Mukk Busster pellets, we wanted to determine if oxygen availability and temperature impact the efficacy of these muck-digesting pellets. We subjected pelleted and non-pelleted sediments to both oxic and anoxic conditions at ambient and ambient+3ºC temperatures in sealed tubes, using the native lake water from which the sediment was collected. We also monitored certain water quality parameters to identify any potential impact of pellet composition on water quality after application. In order to test these

pellets over a larger surface area, we conducted a second experiment using larger containers ("bins"), under ambient room temperatures and darkened environmental conditions. Both the tube and bin results are included in this report.

Methods

Site Descriptions

Three lakes in Newago County, Michigan were identified by the Newaygo County Drain Commissioner's Office for sampling based on prior use of muck-digesting pellets. All three lakes have residential homes on the lakeshore. Hess Lake (Fig. 1; site: N 43.37968, W 85.76893) is a 3.04 km^2 lake with a maximum depth of 7.35 m (24 ft). Brooks Lake (Fig. 2; site: N 43.40285, W 85.75992) is a 1.19 km^2 lake with a maximum depth of 7.92 m (26 ft) and a public boat launch. Pickerel Lake (Fig. 3; site: N 43.46123, W 85.80412) is a 1.29 km^2 lake with a maximum depth of 22.25 m (73 ft) and was accessed via an overnight camp swimming beach.

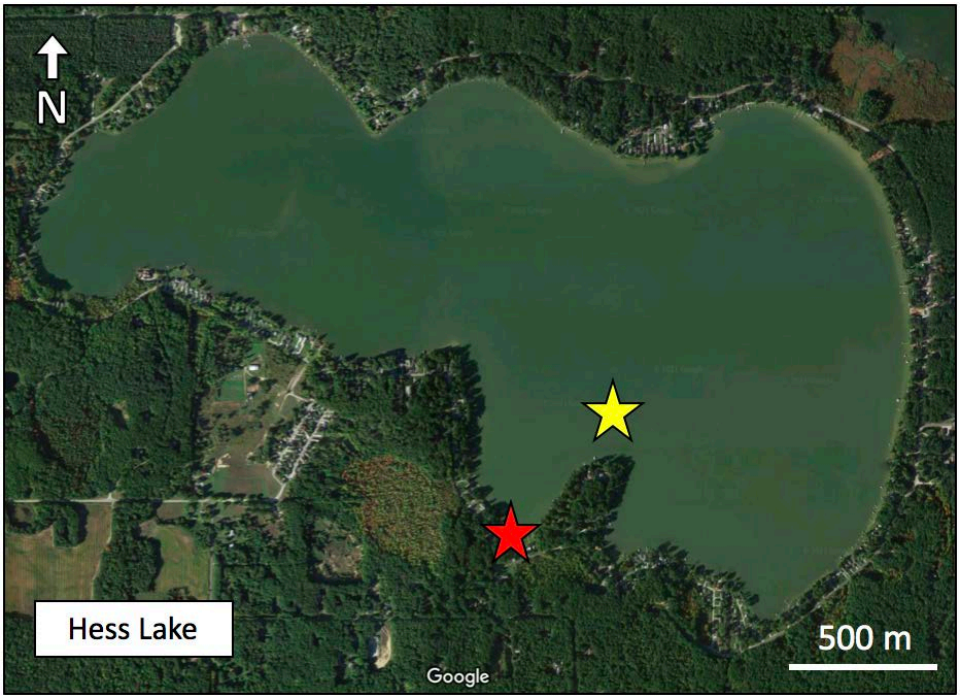

Fig. 1. Hess Lake. Yellow star represents sediment sampling site. Red star represents water sampling site off residential boat dock.

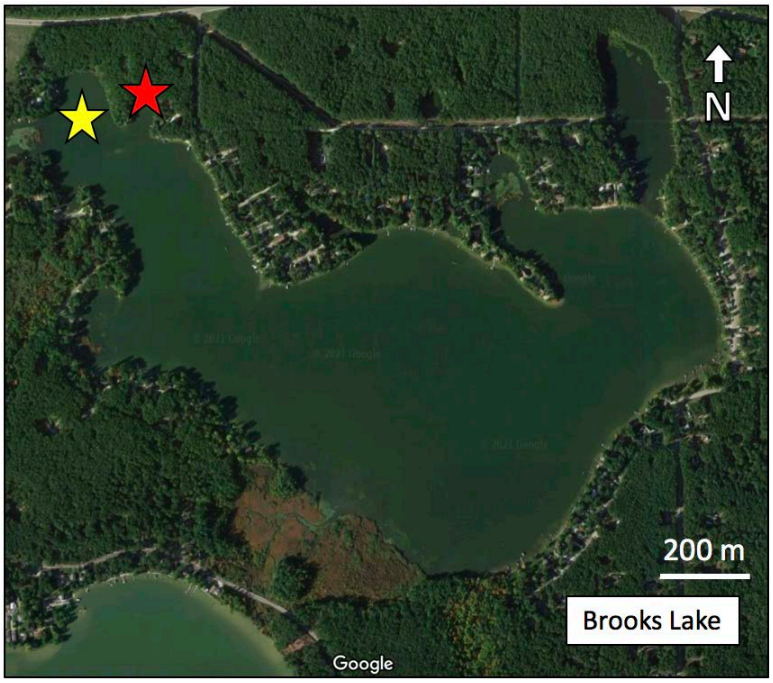

Fig. 2. Brooks Lake. Yellow star represents sediment sampling site. Red star represents boat launch and water sampling site.

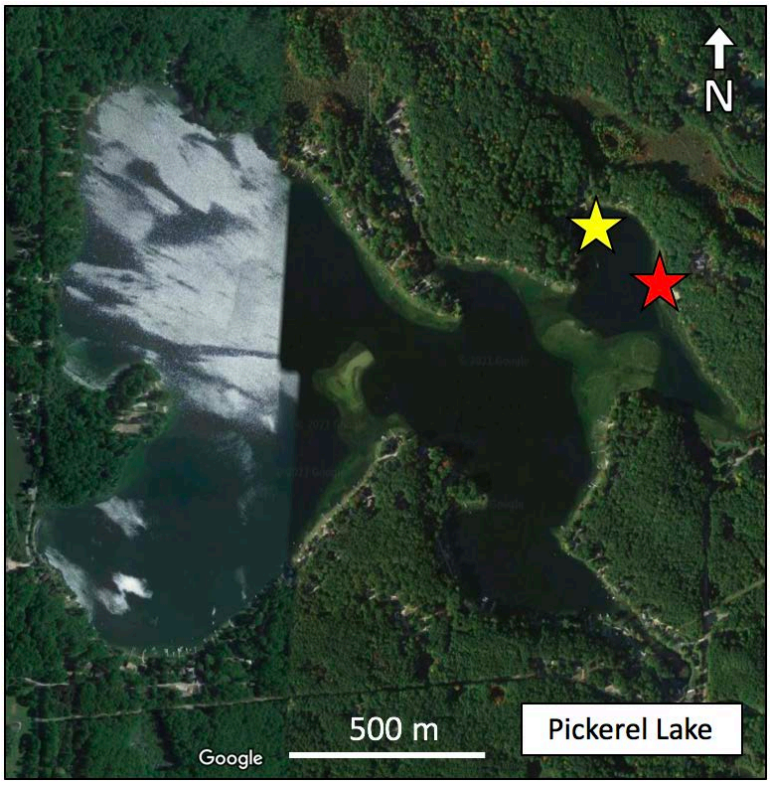

Fig. 3. Pickerel Lake. Yellow star represents sediment sampling site. Red star represents youth camp and swimming beach as well as water sampling site. Note that the lake takes up majority of figure, not just the small dark portion on the right.

Field Methods

All water and sediment sampling occurred on 18 August 2020. Hess Lake water samples were collected from the end of a residential dock and sediment samples were taken from 1.3 m depth (Fig. 1). Brooks Lake water samples were taken from the public boat launch dock and

sediment samples were taken from 2.0 m depth (Fig. 2). Pickerel Lake water samples were taken from the swimming beach dock and sediment samples were taken at 1.4 m depth, near a patch of *Vallisneria* spp. growth (Fig. 3). Sediment sampling sites on all three lakes were accessed via a jon boat, from which photosynthetically active radiation (PAR, LiCor Li-193SA spherical quantum sensor) and Secchi depth (water clarity) were measured. Using a petite Ponar, approximately 38 L of sediment were collected (~10 cm sediment depth) and stored in two 18.9 L buckets and sealed with a lid. All sediments were collected at water depths of ~1-2 m. Eight 18.9 L buckets of surface water were collected from a dock near the jon boat launch and sealed on location. Dissolved oxygen (DO), pH, temperature, and specific conductance (SpCond) were measured at the water sampling site with a YSI 6600 sonde, prior to water collection. The sediment and water were transported back to the laboratory for processing within 6 hr of collection.

Laboratory Methods

Once water and sediment buckets were brought to the lab, the lids were opened slightly to allow for airflow and acclimation to room temperature (~22°C). Water from each lake was sampled for total phosphorus (TP) and alkalinity (Alk), as well as filtered through a 0.45 µm acid-washed filter for soluble reactive phosphorus (SRP), and all stored at 4°C until analysis. Water was also filtered with a 0.45 µm PES filter (FlipMate 100 Sys with pre-filter, Environmental Express- Cole-Parmer, Vernon Hills, IL), pH reduced to <4 with 50% H_2SO_4, and stored at 4°C until delivered to Trace Analytical Laboratories, Inc. (Muskegon, MI) for analysis of dissolved organic carbon (DOC) within two days of sampling. DOC was included in our analyses because we rationalized a reduction in sediment organic matter may manifest itself as DOC or DIC (dissolved inorganic carbon). Ideally, we would have measured all forms of DIC in the water column but this was beyond the scope of our study, so we focused solely on dissolved CO_2 (see below).

The bucketed lake sediments were allowed to settle overnight and then any excess overlying water was removed using a peristaltic pump and discarded. Our experimental set-ups (see below) were completed one lake at a time to avoid contamination of sediment or water among lakes. The two buckets of sediment collected from each lake were combined and mixed

thoroughly. Initial sediment samples for each lake were removed and stored in zip-seal bags at 4°C until processing for percent organic matter (%OM, see below).

Sediment tube experiment. The sediment tubes used for this experiment were clear PVC (25 cm height x 7.5 cm diameter) with punch-out caps that allowed for minimal free exchange of gases (Fig. 4A). We added 200 mL of sediment (surface area of approximately 0.0044 m^2) and 650 mL of water to each tube. All tubes (n=16 per lake) were evenly distributed between two growth chambers that were maintained at either the ambient temperature at the time of collection: 25.8°C; n=8 tubes) or ambient +3ºC: (28.8°C; n=8 tubes). Both growth chambers were set with a 13.75:10.25 light: dark cycle corresponding with western Michigan daylight in August, and had similar average PARs of 40 µmol/m^2/s (ambient) and 41 µmol/m^2/s (ambient +3ºC), mimicking the light levels at the sediment surface. In addition to manipulating temperature, tubes were further divided evenly within each growth chamber into anoxic (bubbled with 5% CO_2 mix [for pH control] with N_2) or oxic (bubbled with air) groups. For the third experimental factor, presence of pellet, tubes were divided evenly into a group receiving muck digesting pellets and a non-pelleted (control) group. This resulted in a combination of three experimental factors (temperature × DO level × presence of pellet), each with two levels (ambient vs. ambient +3ºC; anoxic vs. oxic; pellet present vs. absent), with n=2 tube replicates for each of the three lakes.

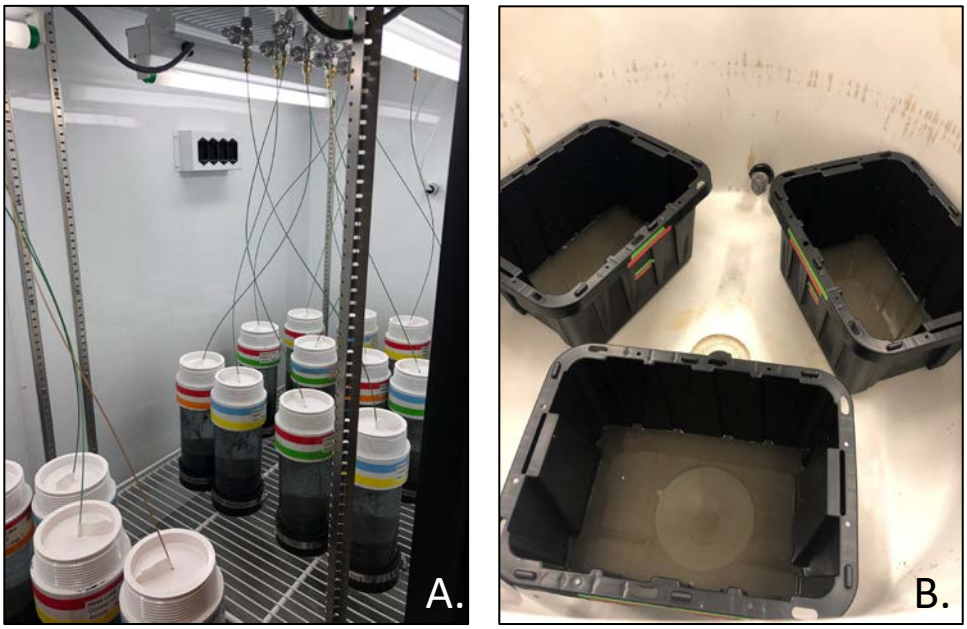

Figure 4. Panel A (left). Sediment tube set-up in growth chamber with gas lines through each knock-out cap in place; tube diameter = 7.5 cm. Panel B (right). Replicate bins for one lake-

either pellet or control. All bins remained open to the air for the whole duration of experiment. Bin interior dimensions at bottom = 0.40 m × 0.29 m.

Sediment bin experiment. Generic 45.4 L storage bins (with the no lids, Fig. 4B) were used to test the pellet muck digestion on a sediment surface area larger than the tubes. We used six bins per lake; three control bins (no pellet) and three with pellet addition. We added ~1420 mL of sediment to each bin (a depth of 2.54 cm, surface area of 0.1204 m^2) and then added 9.5 L of unfiltered lake water to each bin. Each bin was then stirred by hand using a long wooden dowel to allow for even settling of sediment across the bottom of the bin. Bins were maintained at room temperature: ~17°C for the first week of the study and then ~19-22°C for the rest of the duration; however, there was one 2-day temperature drop to ~17°C at the end of October. Light was not regulated for the bins and they were kept under dark conditions for the majority of the study. The bins were not bubbled but one 8-second stirring of the overlying water in each bin was conducted 5-7× per week to represent wind/wave/animal disturbance. A separate wooden dowel was used for each lake/ treatment combination (i.e. one dowel for all three control bins per lake and one dowel for all three pelleted bins per lake).

Both experiments. After the initial sediment and water set-up, all tubes and bins were allowed to settle overnight prior to pellet additions. All extra water from the lake sampling was sequentially filtered through 1.0 µm and 0.2 µm filters, and then stored in buckets at the same temperature as the bins mentioned above until needed for water replacement in the tubes at 4 weeks or for addition to the bins to account for loss due to evaporation.

The muck digesting pellets used for this experiment were Mukk Busster from Cygnet Enterprises, Inc. (Flint, MI) and were donated for this purpose without expectation of reciprocity. The pellets are stated to release bacteria as well as the enzymes lipase, protease, amylase, and cellulase to break down excess organic matter and have been used in regions of all three lakes in the past. The surface area per pellet recommended by the pellet company (one pellet per 0.46 m^2 of lake surface) was much larger than the surface area of our tubes. The smallest practical and repeatably divisible size using a pill cutter for our study was ¼ of a pellet. One-quarter pellet was added to each tube and seven whole pellets were added to each bin, which equated to generally similar degrees of over-application in the tubes and bins (26× and 28×, respectively). Two tubes (Hess Anoxic+3-Rep 1, and Pickerel Anoxic+3-Rep 2) and two bins (Pickerel Pellet-Rep 1 and

Pickerel Pellet-Rep 3) were re-prepared the morning of pellet placement due to set up issues. Then the pellets were introduced and gas bubbling (tube experiment only) commenced.

Sampling Regime

Sediment tube experiment. All tubes were subsampled at 4 weeks and 8 weeks, utilizing the following methods. All control tubes were sampled first, followed by all pelleted tubes, one lake at a time. Between each tube sample, all equipment was cleaned with 70% isopropyl alcohol to minimize bacterial transfer and 10% HCl to minimize phosphorus contamination. On each sampling date, *in situ* DO was measured and then the surface water was removed with a peristaltic pump with minimal disturbance of the sediment surface (~1 cm of water remained) and transferred to a clean beaker. Water quality parameters were measured on the transferred water with a YSI and then the water was subsampled for TP/Alk (250 mL), SRP (20 mL), and DOC (100 mL), as was done with the initial lake samples (see above). Two sediment cores were sampled from each tube by coring with a modified 15 mL centrifuge tube, for a total removed volume of approximately 20 cm^3 from each tube. One core was transferred to a sterile 60 mL centrifuge tube and then frozen at -80°C for bacterial analysis; the second core was transferred to a pre-ashed and weighed ceramic crucible for processing of ash-free dry mass (AFDM) and %OM (see below). After the 4-week sediment subsampling, 650 mL of corresponding filtered lake water was gently added back to the tubes with the peristaltic pump to minimize disturbance. All tubes were allowed to settle at least one hour and then a new ¼-piece of pellet was added to all "pellet" cores and the bubbling was restarted. All environmental and bubbling conditions for tubes were continued for the second 4 weeks. Following the 8-week sampling, all remaining sediment from each tube was transferred to a zip-seal bag for a second analysis of AFDM and %OM.

Sediment bin experiment. Sediment in bins was sampled only at the end of the experiment (see below). However, at 4 weeks, *in situ* DO was measured and a portion of water was removed by peristaltic pump to measure pH and SpCond with the YSI. This water was then returned, along with 3000 mL of filtered lake water, to compensate for evaporative losses. The water and sediment in each bin were mixed thoroughly and then allowed to settle overnight. All control bins were sampled before cleaning equipment and proceeding to the pelleted bins to prevent bacterial cross contamination (as with the tubes described above). After settling, seven

new whole pellets were added to each of the pellet treatment bins. The bin stirring regime was continued for the rest of the experiment. Another 3000 mL of corresponding filtered lake water was again added at 8 weeks to account for evaporation.

Hess bins were processed at 12 weeks, Brooks bins at 13 weeks, and Pickerel bins at 15 weeks (hereafter referred to as "end timeframe"). After measurement of *in situ* DO, all water was removed with a peristaltic pump, and sampled for TP/Alk (250 mL), SRP (20 mL), and DOC (100 mL), as well as water quality parameters with the YSI. The sediment in each bin was then homogenized and ~15 mL was transferred to a sterile 60 mL centrifuge tube and frozen at -80°C for bacterial analysis (see below). All of the remaining sediment was transferred to a clean 18.9 L bucket and covered loosely while awaiting sediment processing within one week of breakdown. Sediment was transferred in stages to pre-ashed and weighed disposable aluminum cake tins for %OM analysis (see below).

Percent OM and CO_2 Determination. All sediment samples to be analyzed for AFDM were dried at 90°C until constant mass and then ashed at 550°C for 1 hr (Steinman et al. 2017). This included initial lake sediment samples, tube-cored samples at 4 weeks and 8 weeks, entire tube end samples (8 wk), and entire bin end sediment samples (12, 13, or 15 wk).

$$\%OM = [(\text{Dry mass} - \text{Ashed mass})/\text{Dry mass}] * 100$$

All dissolved CO_2 values were calculated utilizing the Millero method (Prieto and Millero 2002, adjusted for freshwater) via the online calculator "Freshwater CO2 Level Calculator" (https://www.hamzasreef.com/Contents/Calculators/CO2LevelFresh.php). This method is based on alkalinity, pH, and temperature.

Bacterial Composition Analysis. Sediment from the 8-week incubator experiment and bin experiment was subsampled and DNA was extracted using a Macherey-Nagel Nucleospin Soil DNA extraction kit (Macherey-Nagel, Bethlehem, PA). Samples were amplified using PCR for the 16S rRNA v4 region using the 515F/806R primer set (Caporaso et al., 2012). The region was amplified using a two-step PCR process with the conditions outlined in the Illumina 16S Amplicon Sequencing Library Preparation protocol (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf).

Sample libraries were pooled and the amplicons were sequenced using a 2 × 250 bp format, along with a 15% spike-in of Phi-X, on the Illumina MiSeq System (Illumina, San Diego, CA). Sequence alignment, chimera removal, taxonomic identification, operational taxonomic unit (OTU) identification, and abundance information was obtained using the program MOTHUR v1.42.3 (Schloss et al., 2009). Briefly, reverse and forward reads were assembled and sequences with ambiguous calls or homopolymers longer than 8 bp were removed. Sequences were aligned to the full Silva bacteria reference database v132. Chimeras were identified using VSEARCH and removed. Sequences were classified based on the RDP classifier trainset18_062020 files provided by MOTHUR, and sequences identified as "chloroplast", "mitochondria", "Archaea", "Eukaryota", and "unknown" were removed. OTUs were binned by clustering via 97% sequence similarity. To avoid bias due to unequal sequencing depth among samples, all sequences were randomly subsampled to 28,000 sequences per sample.

Data Analysis

Data were analyzed separately for each lake due to differences in sediment, water chemistry, macrophytes at sampling site, and human use. Changes in %OM, absolute OM (g), DOC, dissolved CO_2 (hereafter "CO_2"), TP, SRP, Alk, SpCond, and pH were analyzed between lake measurements (as initial values) and measured experimental values for the tubes at 4 weeks and 8 weeks, and for the bins at the end timeframe as described below. Due to the small tube sample size per each set of main factorial combinations, as well as issues with homogeneity of variances and normality, a more robust 3-way analysis (t3way ( ) {WRS2}, Mair and Wilcox 2020) was used. If the 3-way analysis revealed identical significant factors, then applicable 3-way ANOVA post-hoc analyses were conducted on those significant factors to parse out specific comparisons, as there are currently no effective robust 3-way post-hoc analyses. The post-hoc tests utilized for significant 3-way factors included pairwise comparisons using estimated marginal means (emmeans_test ( ) {emmeans}, Lenth 2020), effect of treatment factors, and simple main effects using 1- and 2-way tests on interaction terms.

With only the pellet treatment for the sediment bin experiment, t-tests or Kruskal-Wallis tests were used to identify any significant differences between pelleted and control bins for changes in SpCond and pH at 4 weeks and %OM, absolute OM(g), DOC, CO_2, and water quality parameters at the end timeframe. Chloride also was measured for the bin experiment and

compared between pelleted and control bins only for the end timeframe. All analyses were conducted in R (v 4.0.3, The R Foundation for Statistical Computing).

Stacked bar plots were created to identify bacteria genera whose relative abundance surpassed at least 1.0% of the total community for the 8-week incubator sediment samples and the bin sediment samples. Amplicon data from the 8-week incubator sediment samples and bin samples also were examined with Principal Coordinates Analysis (PCoA) of OTUs using the R statistical package phyloseq v1.34.0 (McMurdie and Holmes, 2013). Genetic distances among each of the samples were calculated using the Bray-Curtis method (Bray and Curtis, 1957) in phyloseq, which included a square root transformation and Wisconsin double standardization for both the 8-week incubator and bin experiments. A permutational analysis of variance (PERMANOVA) using the distance matrix was performed using the adonis function in vegan v2.5-7 (Oksanen et al., 2019) to examine significant differences in centroid location associated with lake, treatment, dissolved oxygen, and temperature for the incubator experiment, and lake, treatment, and the interaction between lake and treatment for the bin experiment.

Measures of alpha diversity for genus-level bacterial OTUs were obtained to assess OTU richness (i.e., the number of bacterial OTUs present in each sample), and OTU evenness (i.e., the relative distribution of each OTU for each sample). These were quantified by subsampling each sample with replacement to a minimum sequence depth (20,000 reads) 100 times to gain estimates of species abundance. Observed richness and evenness, based on inverse Simpson's index (Sun, 1991), were quantified for each sample, using phyloseq v1.34.0 (McMurdie and Holmes, 2013). A 3-way ANOVA was used to examine the effects of treatment, temperature, and dissolved oxygen, and their interactions on bacterial community richness and evenness estimates for the 8-week incubator experiment. A 2-way ANOVA was used for the bin experiment to determine the effect of lake, treatment, and the interaction between lake and treatment on richness and evenness estimates.

Results and Discussion

Lake Water Quality

Water quality, with the exception of PAR at sediment surface and TP in the water column (Tables 1 and 2), was similar among lakes. Hess Lake had higher PAR at the sediment surface than Brooks and Pickerel Lakes, despite being sampled at generally similar depths and having

similar Secchi depths. Hess Lake surface water TP was 2× and 4× greater than Brooks and Pickerel Lakes, respectively, but surface water SRP measurements were below detection in all lakes. Chlorophyll concentrations were quite high, suggestive of eutrophic conditions in all three lakes (Table 1).

Table 1. Lake physical water quality parameters measured on 18 August 2021 from a single site per lake (see Figs. 1-3). PAR = photosynthetically active radiation, DO = dissolved oxygen, SpCond = specific conductance.

	Secchi Depth (m)	PAR at sampling depth (µmol/m²/s)	Site Depth (m)	DO (mg/L)	pH	Temp (°C)	SpCond (µS/cm)	Turbidity (NTU)	Chl (µg/L)
Hess	0.7	200	1.3	9.03	8.82	24.66	296	45.0	54.1
Brooks	0.8	30	2.0	9.29	8.76	26.29	308	22.6	47.9
Pickerel	1.1	54	1.4	9.15	8.58	26.33	319	6.5	46.9

Lake sediment %OM measurements (Table 2) were compared among the lakes, but only for the sampling sites, so we cannot account for spatial variability within lakes. Hess Lake had significantly higher sediment %OM (also used as initial %OM for lab studies) than both Brooks and Pickerel Lakes ($p<0.0001$), while Brooks and Pickerel Lake initial sediment %OM was not significantly different from each other ($p=0.456$).

Table 2. Lake water quality parameters measured on 18 Aug 2020. Dissolved CO_2 calculated by the Millero method from lake temperature, pH, and alkalinity. Average sediment %OM are means of 3 subsamples from the sediment grab at one site per lake. TP = total phosphorus, SRP = soluble reactive phosphorus, DOC = dissolved organic carbon; %OM = percent organic matter.

	TP (mg/L)	SRP (mg/L)	Alkalinity (mg/L)	DOC (mg/L)	Dissolved CO_2	Avg. Sediment %OM
Hess	0.042	<0.005	128	13	0.21	47.8 ± 0.3
Brooks	0.024	<0.005	132	13	0.25	17.3 ± 0.2
Pickerel	0.009	<0.005	144	12	0.46	17.6 ± 0.7

Changes in Organic Matter

Each of the three factors we examined as possible influences on organic matter mineralization (pellet, temp, and DO) had two levels (Table 3). We examined their effects with a 3-way test, as described below.

Table 3. Factors, levels, and abbreviations in the tube experimental design.

Factor	Levels	Abbreviations
Pellet	Present Absent (Control)	PT C
Temperature (Temp)	Ambient Ambient plus 3°C	Amb +3
Dissolved Oxygen level (DO)	Anoxic Oxic	AN OX

Percent Organic Matter: Tube Experiment. Unexpectedly, percent sediment organic matter increased in two of the three lakes, in both the control and pellet treatments (Fig. 5, Table B1. Regardless of whether %OM increased or decreased, there was no significant effect of pellets on tube %OM for any lake. In addition, neither temperature nor dissolved oxygen content had any statistically significant impact on change in %OM in either Hess Lake or Pickerel Lake sediment based on the 3-way analyses (Fig. 5, Table A1). Brooks Lake temp × DO interaction term was significant at 4 weeks and 8 weeks ($p=0.039$, $p=0.004$), but again the presence of pellets had no effect, as %OM accumulation was the same regardless of temperature or DO treatment (Fig. 5, Table A1). Despite the interaction term being significant for DO only ($p=0.017$) at 4 weeks, there were no significant pairwise comparisons. At 8 weeks, temp × DO had an effect on C tubes only ($p=0.011$).

Absolute Amounts of Organic Matter: Tube Experiment. The overall trends for absolute organic matter were, as expected, the same as for %OM. However, it is worth noting that the increases in absolute amounts of organic matter were small, especially in Hess Lake (Fig. 6). Statistical results were very similar to those for %OM (Table A2).

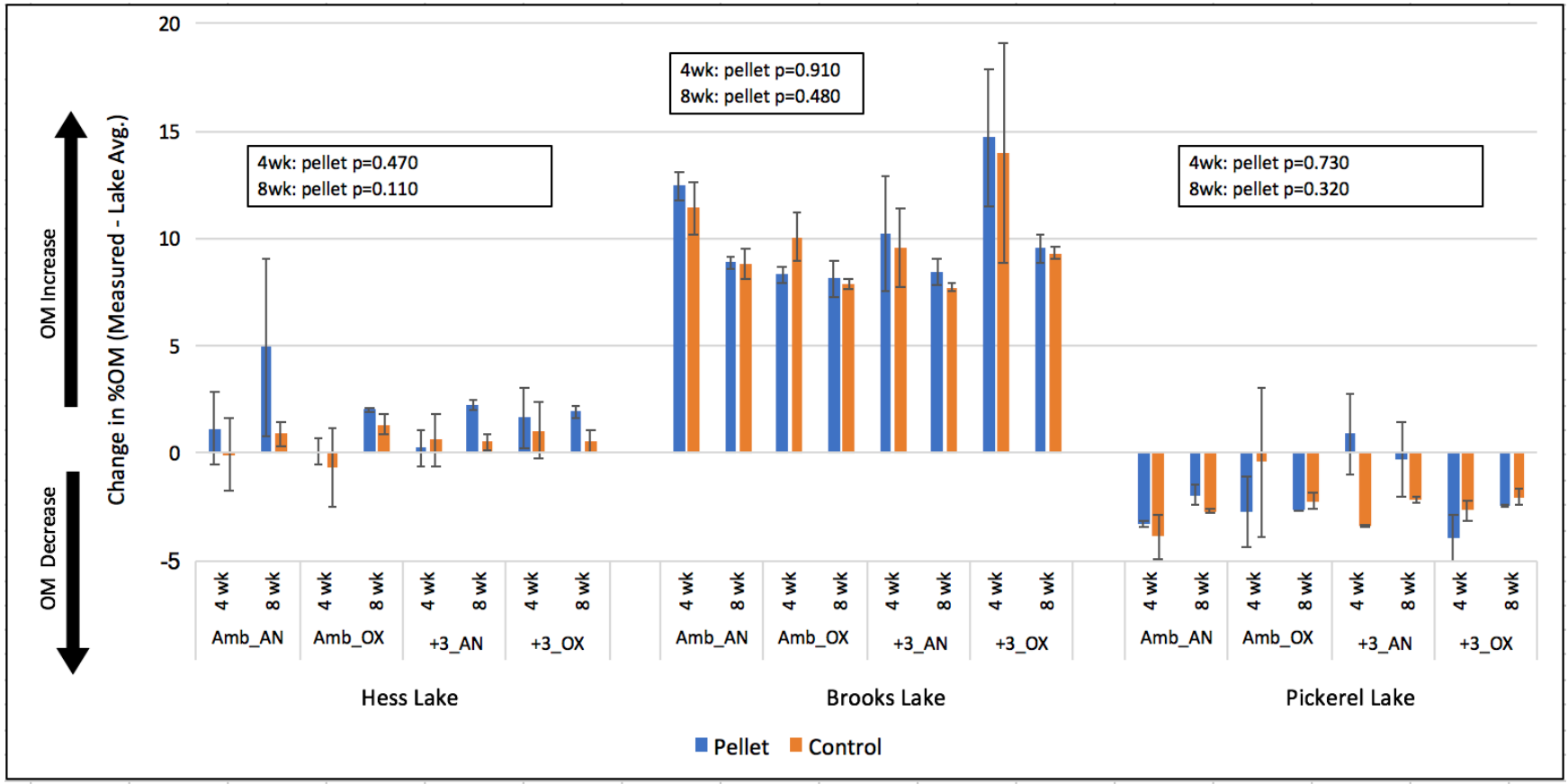

Fig. 5. Changes in percent organic matter for all experimental combinations of pellet, temp, and dissolved oxygen concentration at four and 8 weeks in the tubes. Bars above the horizontal axis represent increases in %OM while bars below the axis represent decreases in %OM. Amb = ambient temperature, +3 = ambient temperature plus 3ºC, AN = anoxic, OX = oxic.

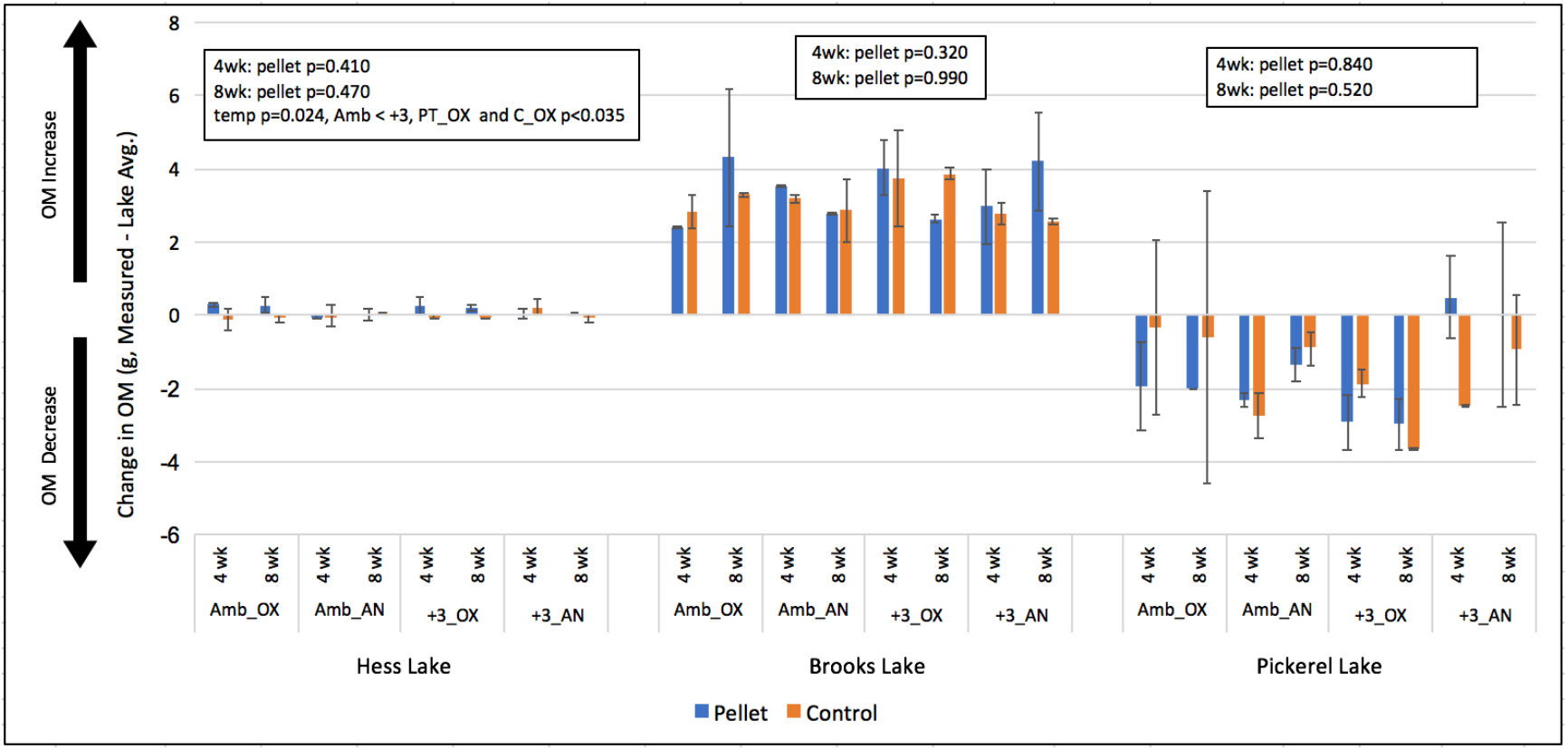

Figure 6. Changes in absolute levels of organic matter (OM, in g) for all experimental combinations of pellet, temperature, and dissolved oxygen level over four and 8 weeks in the tubes. Bars above the horizontal axis represent increases in OM(g) while bars below the axis represent decreases in OM(g). PT= pelleted, C= control, Amb = ambient temperature, +3 = ambient temperature plus 3°C, AN = anoxic, OX = oxic.

Percent Organic Matter: Bin Experiment. There was no significant difference in the change of sediment %OM between pelleted and control treatments in the bin experiment for any lake (Table B1). Even with an over-application of pellet to surface area (28× recommended application), these pellets were not effective in reducing %OM, at least within the two/three-month time period and under the present experimental conditions (Fig. 7).

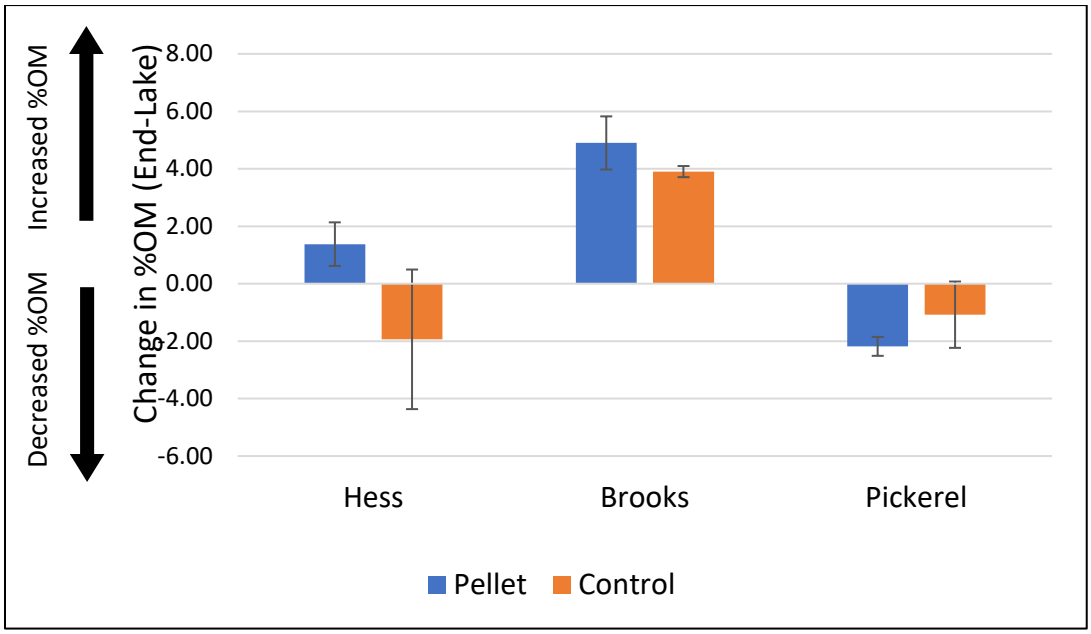

Figure 7. Changes in percent organic matter (%OM) between the lake sediment and ending sediment in the bins. There were no significant differences between the changes in pelleted and control bins for any lake.

Absolute Amounts of Organic Matter: Bin Experiment. Similar to the results for %OM, there were no significant differences between the changes in pelleted and control bins for any lake (Fig. 8, Table B2).

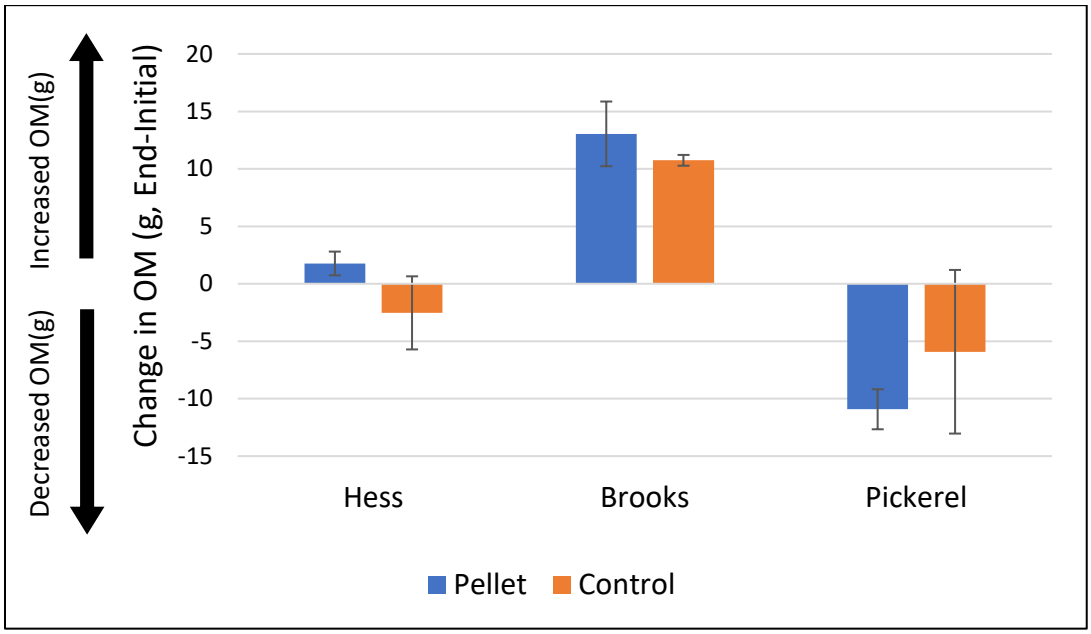

Figure 8. Changes in absolute amounts of organic matter (OM, in g) between the lake sediment and ending sediment in the bins.

Despite the differences in organic matter changes among lakes, the overall responses in organic matter were very similar regardless of whether the experiments took place in the tubes or the bins: we observed relatively small changes in Hess Lake, increases in Brooks Lake, and declines in Pickerel Lake. But in all cases, the presence of pellets had no significant effect on changes in organic matter.

Fate of Organic Matter

Our expectations at the outset were that if the pellets were effective at reducing organic matter, the reduction would result in an increase in either dissolved organic carbon or dissolved inorganic carbon, as basic physics states that mass must be conserved over time. Our resource constraints precluded our tests being conducted in an entirely closed system, so the CO_2 results must be interpreted with caution (see below).

Dissolved Organic Carbon: Tube Experiment. If the pellets were mineralizing organic matter, then one possible result would be an increase in DOC. However, there was no indication that DOC concentration was greater in the pellet than control treatments (Fig. 9, Table A3). Indeed, the only statistically significant result regarding the pellet treatment revealed greater DOC in the control than pellet treatment in Hess Lake, but with Amb_OX tubes only ($p<0.001$).

At 8 weeks, there was no significant effect of pellets in all three lakes (Fig. 9), although DO was significant (Hess $p=0.001$, Brooks $p=0.001$, Pickerel $p=0.001$), with greater DOC under anoxic than oxic conditions (Fig. 9, Table A3). This is likely a result of CO_2 being mixed with N_2 in the anoxic treatment (see below). Several interaction terms were statistically significant, again mostly involving DO concentrations (Table A3).

Dissolved CO_2: Tube Experiment. Organic matter decomposition also could release dissolved inorganic carbon. Ideally, we would measure DIC via an in-line sensor in a closed, flow-through system, but that was not feasible given the scope of our experiment. Instead, we calculated CO_2 in the water, keeping the head space in the tubes as small as possible. In addition, the anoxic treatments involved the bubbling of N_2 gas combined with CO_2 for pH buffering, which obviously could confound the interpretation of CO_2 concentrations in the water column.

We did not detect any significant effect of the pellet treatment on the change in tube dissolved CO_2 for any lake (Fig. 10). However, there was a clear effect of DO, with greater CO_2 concentrations in all three lakes in anoxic vs. oxic treatments (all $p=0.001$; Fig. 10, Table A5). In addition, the effect of DO was greater at 4 wk than at 8 wk. A number of interactions was significant but none had a consistent pattern (Table A5). At 8 weeks, all three lakes had significant robust 3-way results also for the single factor DO on tube CO_2 (all $p=0.001$). These results are consistent with those for DOC, where increases in CO_2 in anoxic tubes are most likely due to the bubbling of $N_2 + CO_2$ gas into the water column.

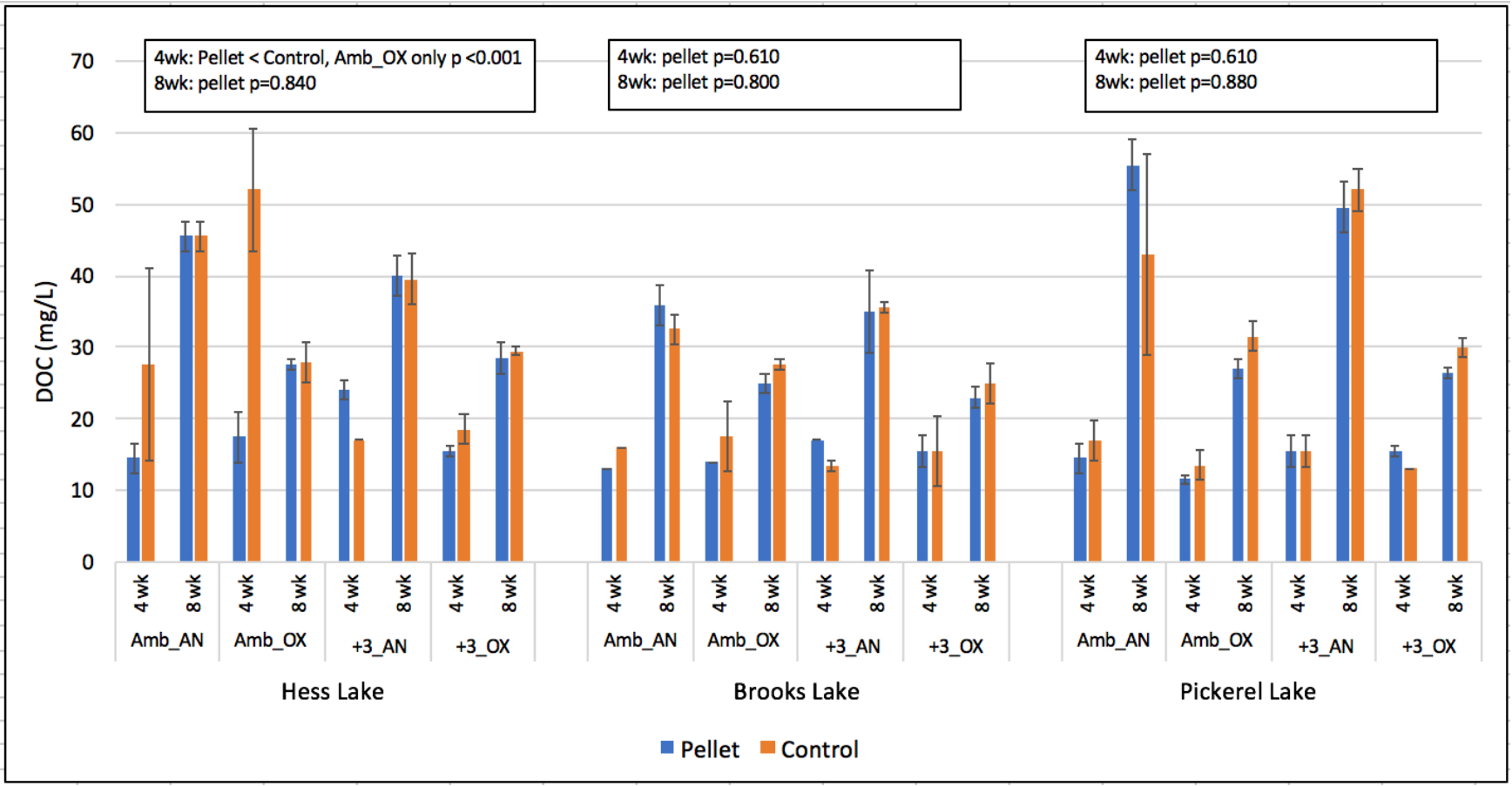

Fig. 9. Dissolved organic carbon (DOC) for all experimental combinations of pellet, temperature, and dissolved oxygen concentration for four and eight weeks in the tubes. Amb = ambient temperature, +3 = ambient temperature plus 3ºC, AN = anoxic, OX = oxic.

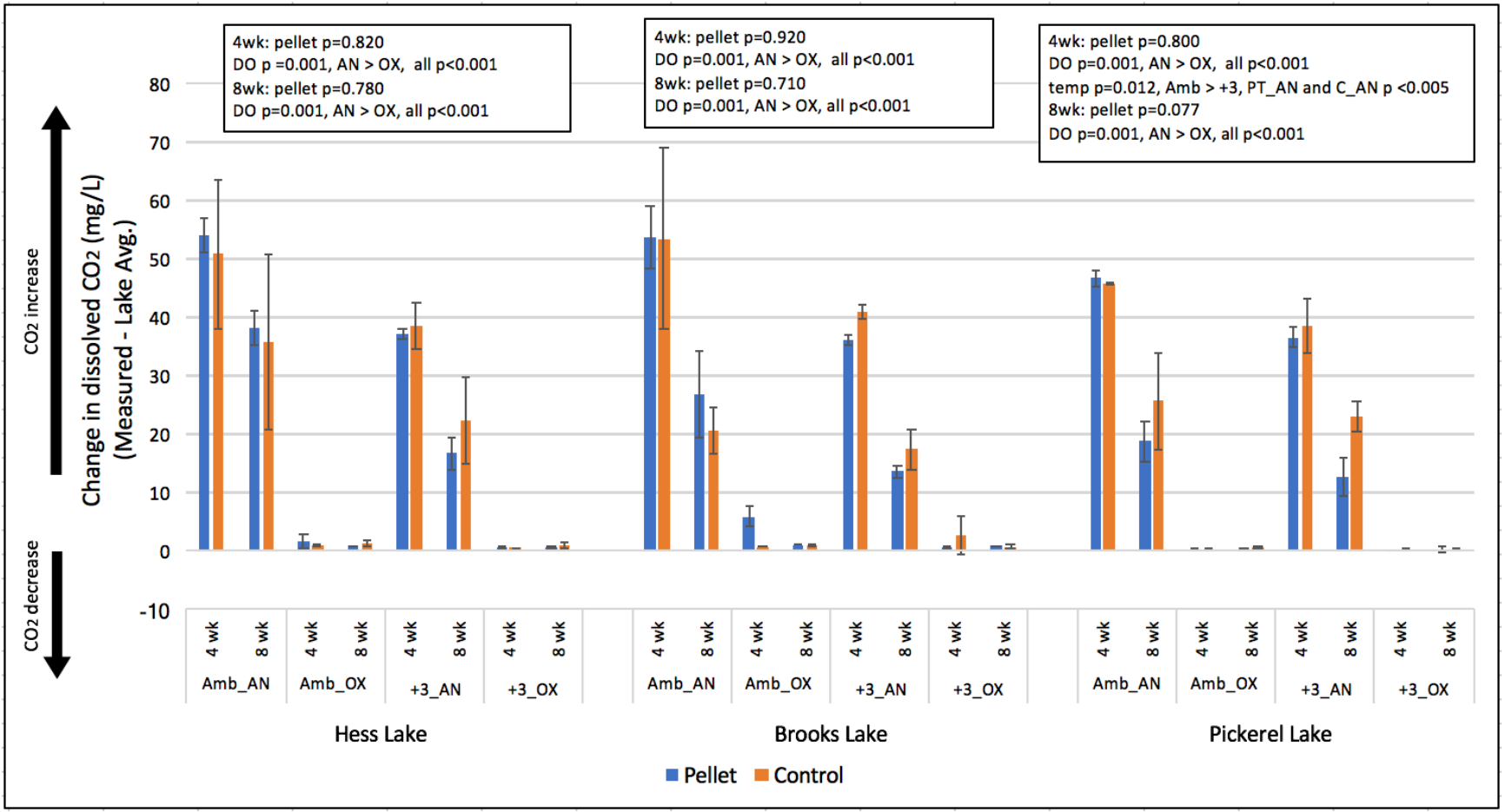

Figure 10. Changes in dissolved carbon dioxide (CO_2 in mg/L) for all experimental combinations of pellet, temperature, and dissolved oxygen level over four and eight weeks in the tubes. Bars above the horizontal axis represent increases in CO_2 while bars below the axis represent decreases in CO_2. Amb = ambient temperature, +3 = ambient temperature plus 3°C, AN = anoxic, OX = oxic.

Dissolved Organic Carbon: Bin Experiment. The bin experiments did not include a DO treatment, so the artefact of bubbling CO_2 was not a concern. The only significant difference in the change of DOC between pelleted and control bins (Fig. 11) was in Pickerel Lake ($p<0.0001$, $t = -6.4\ e+10$, and a $df= 4$, ignoring normality issues as their standard deviations were zero; see Table B3). However, mean changes in DOC were greater in the pelleted bins in all lakes, possibly indicating greater mineralization of organic matter (but not supported by the OM results: Figs. 7, 8). It is possible that a longer duration experiment may have seen more of an effect, although the experiments ran the approximate amount of time recommended by the manufacturer in lakes.

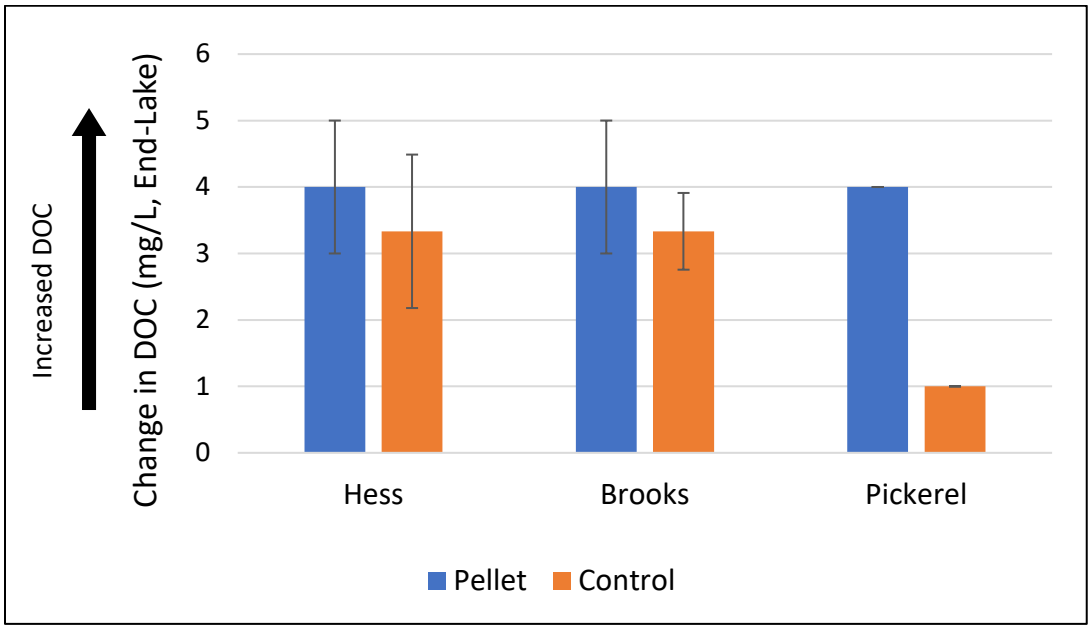

Figure 11. Changes in dissolved organic carbon (DOC, in mg/L) between the lake sediment and ending sediment in the bins. There were no significant differences between the changes in pelleted and control bins for any lake. Pickerel pellet and control DOC had standard deviations of zero.

Dissolved CO_2: Bin Experiment. Similar to DOC, there was no significant difference in change of CO_2 between pelleted and control bins for any lake (Fig. 12, Table B4). These results are consistent with the finding that the increases in both DOC and CO_2 observed in the tube experiments were due to the bubbling effect and not due to mineralization of organic matter.

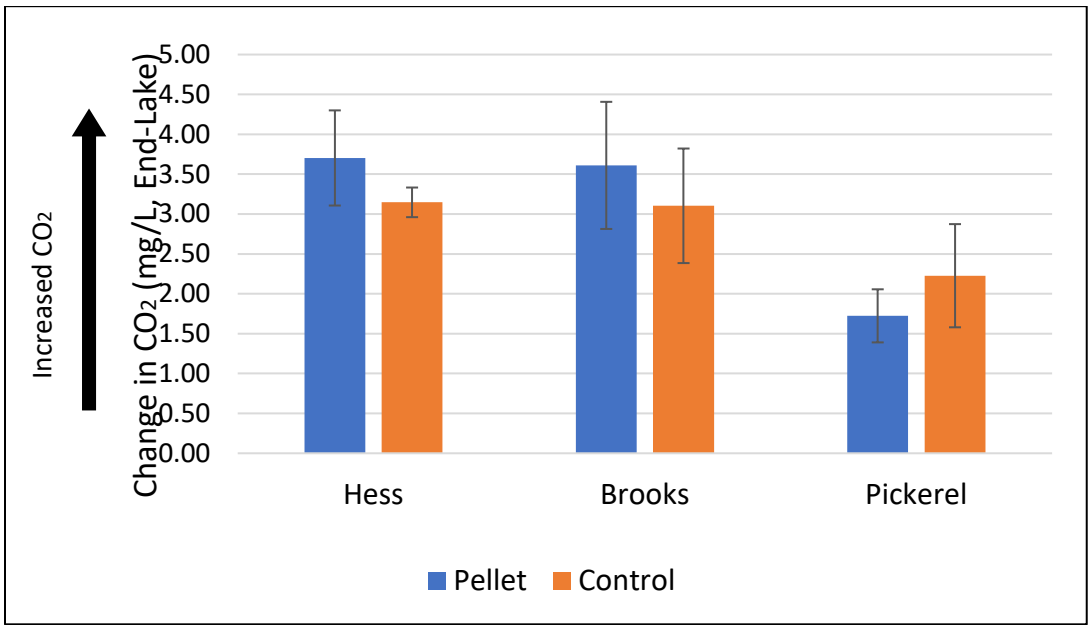

Figure 12. Changes in dissolved CO_2 (mg/L) between the lake sediment and ending sediment in the bins. There were no significant differences between the changes in pelleted and control bins for any lake.

Differences in Lake Responses

Although the pellets had no effect on changes in organic matter in any of the three tested lakes, there were clear and consistent differences *among* the lakes in how organic matter responded (Figs. 5, 6). As a consequence, we investigated differences in water quality among the lakes, as well as changes in water quality over the experimental period, to determine if certain parameters may explain these differences. We focused on water quality changes in the bins, to avoid artefacts created from the bubbling of gases in the tubes.

As seen in Table 2, Hess Lake was the outlier of the three lakes with respect to initial TP and %OM concentrations. However, Hess Lake also had the least net change in OM over the course of the experiments, suggesting that neither initial TP nor %OM accounted for the different patterns in OM among the lakes. In contrast, the change in TP concentration in the bin water column was inversely related to the changes in OM, with TP in the Pickerel bin increasing over time and the water TP in Brooks declining (Fig. 13). We did not measure the initial sediment chemistry given that our focus was on the effect of pellets and OM, but it is possible that the different results we observed among the lakes was attributable to sediment chemistry. In

this case, increased mineralization of OM in Pickerel, independent of pellets, may have accounted for the decline in OM and release of phosphorus to the water column. It is unlikely that the increase in Pickerel TP is due to increased plankton growth, simply because SRP was still quite high (Fig. 14), and we would expect actively growing plankton to draw the SRP concentrations down to levels seen in the initial samples, which were below detection (Table 2).

None of the changes in the other water quality parameters was consistent with the changes in OM among lakes (Figs. 14-17, Tables B5-8). However, overlying water in the pellet treatments had significantly greater specific conductance (Fig. 16, Table B7) and chloride concentrations (Table B8), but not pH (Fig. 17, Table B9), than control treatments.

We attribute these increases to the contents of the pellets themselves, given that they were introduced at a much greater dosage than recommended due to the small size of our containers. It is possible the high chloride concentrations could have significant negative impacts to biota in lakes. All the bins with pellets had mean chloride concentrations far exceeding the 230 mg/L chronic toxicity threshold for freshwater and also exceeded the 860 mg/L acute toxicity threshold (NaCl; USEPA 1988), although when normalized by our overdosing using Hess Lake as an example, the overall effect is an estimated 16.3 mg/L increase in chloride (dividing the observed increase in chloride of 914 mg/L (943 mg/L mean pelleted minus 29 mg/L mean control) by 56 (28× the recommended dose *2 applications)). It seems unlikely dosing at the recommended levels would result in Cl toxicity, although homeowners should be careful to follow label recommendations, and not overdose their lakes.

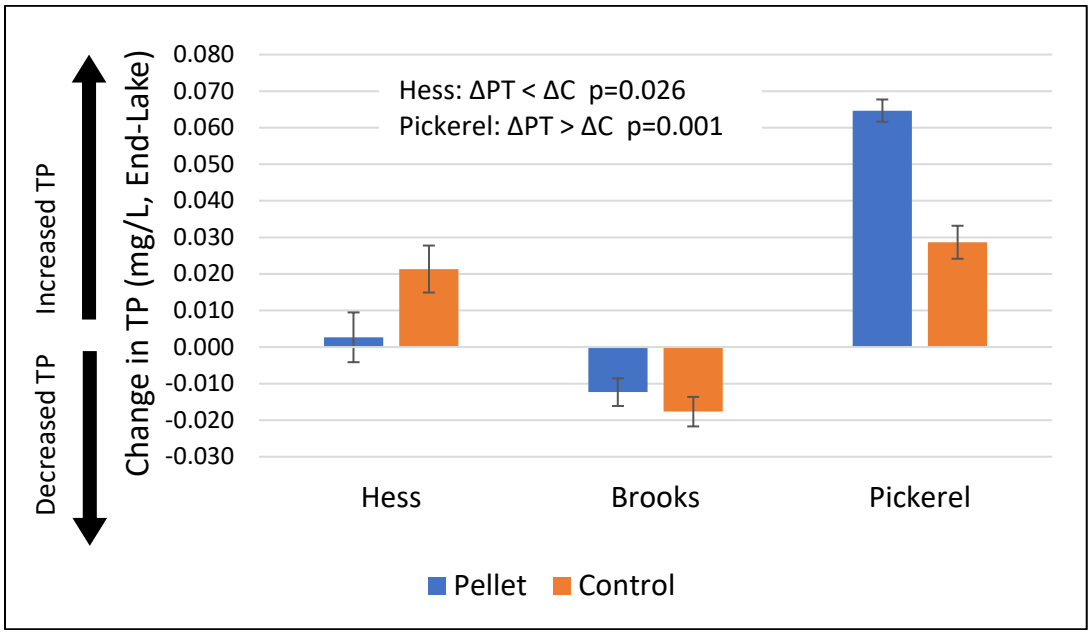

Figure 13. Changes in total phosphorus (TP, in mg/L) between the lake sediment and ending sediment in the bins. The change of TP in Hess Lake pelleted bins was significantly less than the change in the control bins. Oppositely, the change of TP in Pickerel Lake pelleted bins was significantly greater than the change in control bins. Brooks Lake had no significant differences between the change in pelleted and control bins.

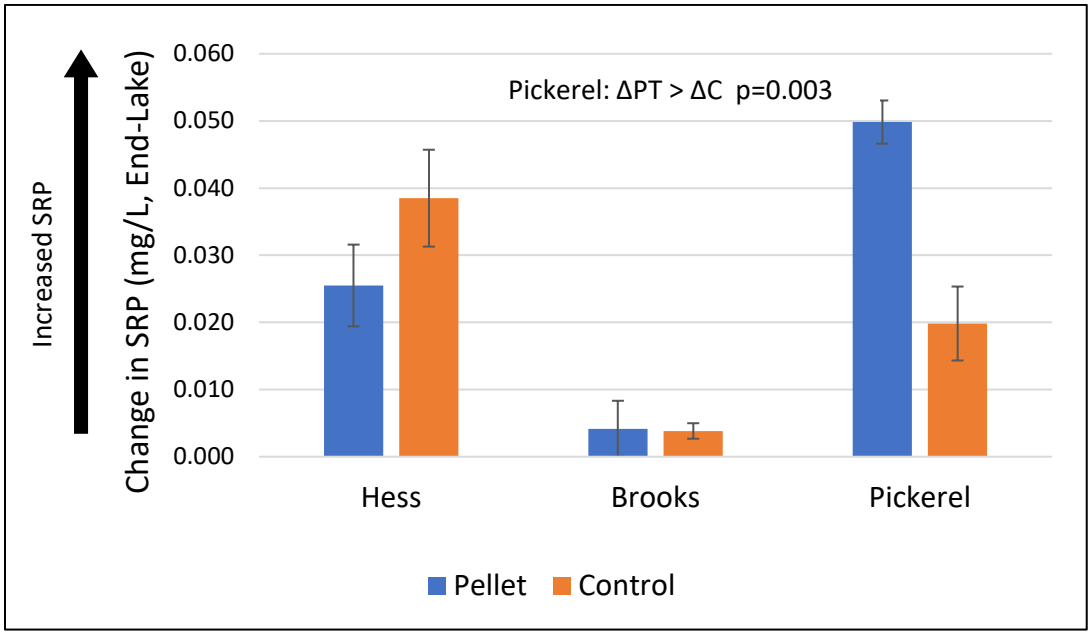

Figure 14. Changes in soluble reactive phosphorus (SRP, in mg/L) between the lake sediment and ending sediment in the bins. The change of SRP in Pickerel Lake pelleted bins was significantly greater than the change in the control bins. Both Hess Lake and Brooks Lake had no significant differences between the change in pelleted and control bins.

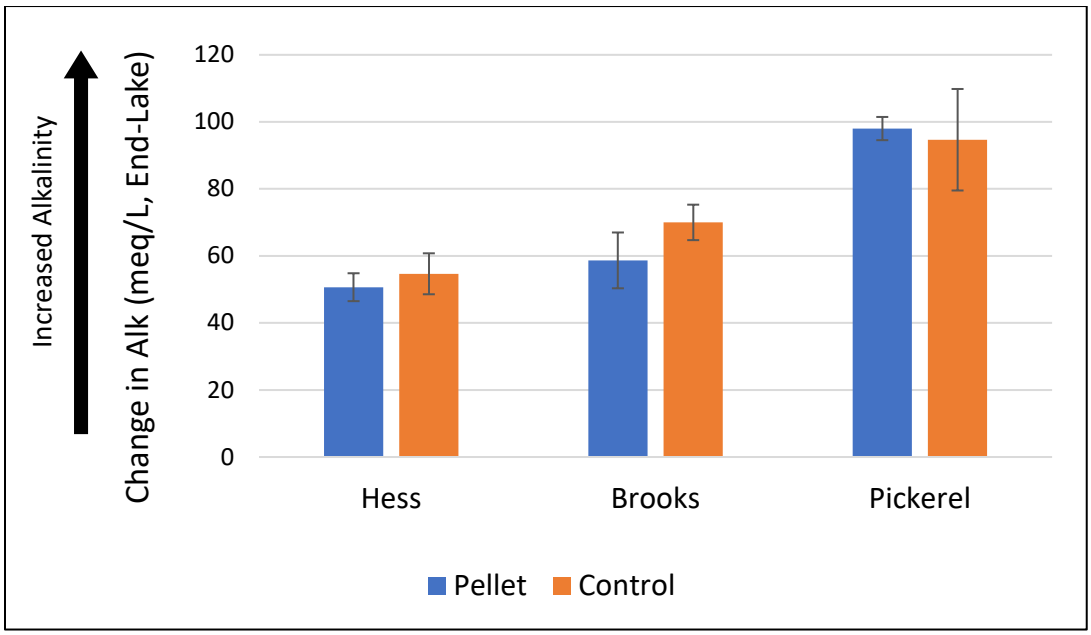

Figure 15. Changes in alkalinity (Alk, in meq/L) between the lake sediment and ending sediment in the bins. There were no significant differences between the changes in pelleted and control bins for any lake.

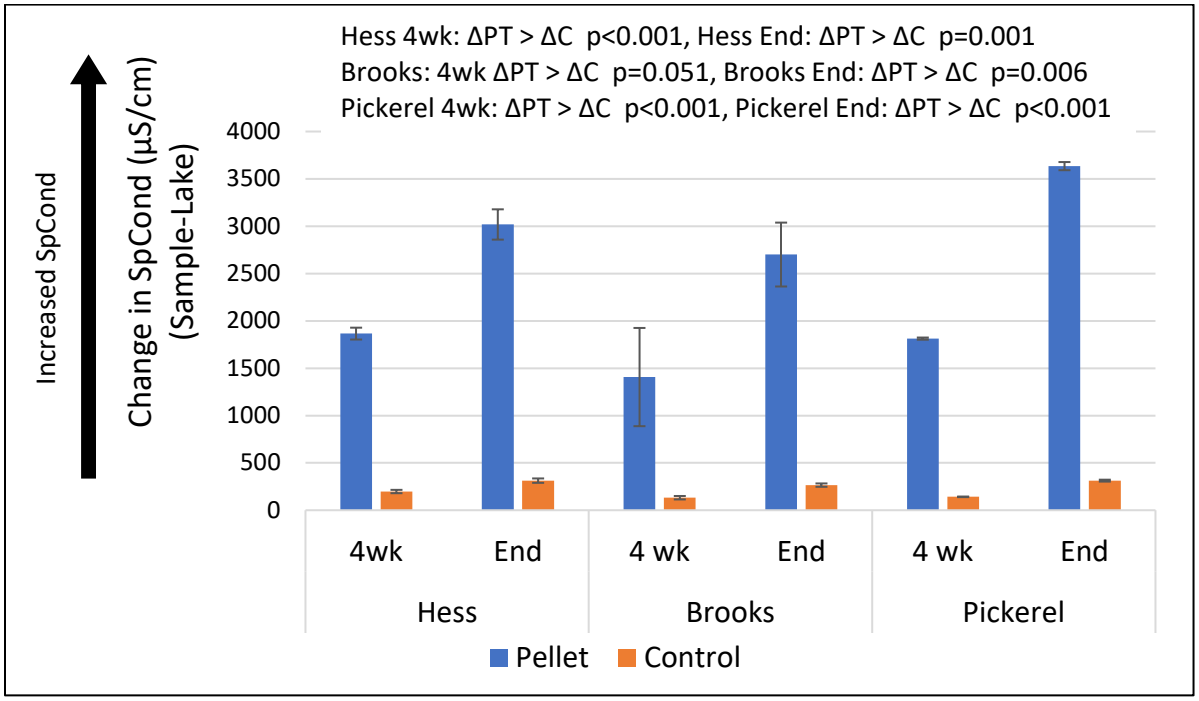

Figure 16. Changes in specific conductance (SpCond, in µS/cm) between the lake sediment and ending sediment in the bins. The change of SpCond was significantly greater in pelleted bins than the change in control bins for all three lakes over the first four weeks (4wk) and the entire experiment duration (End).

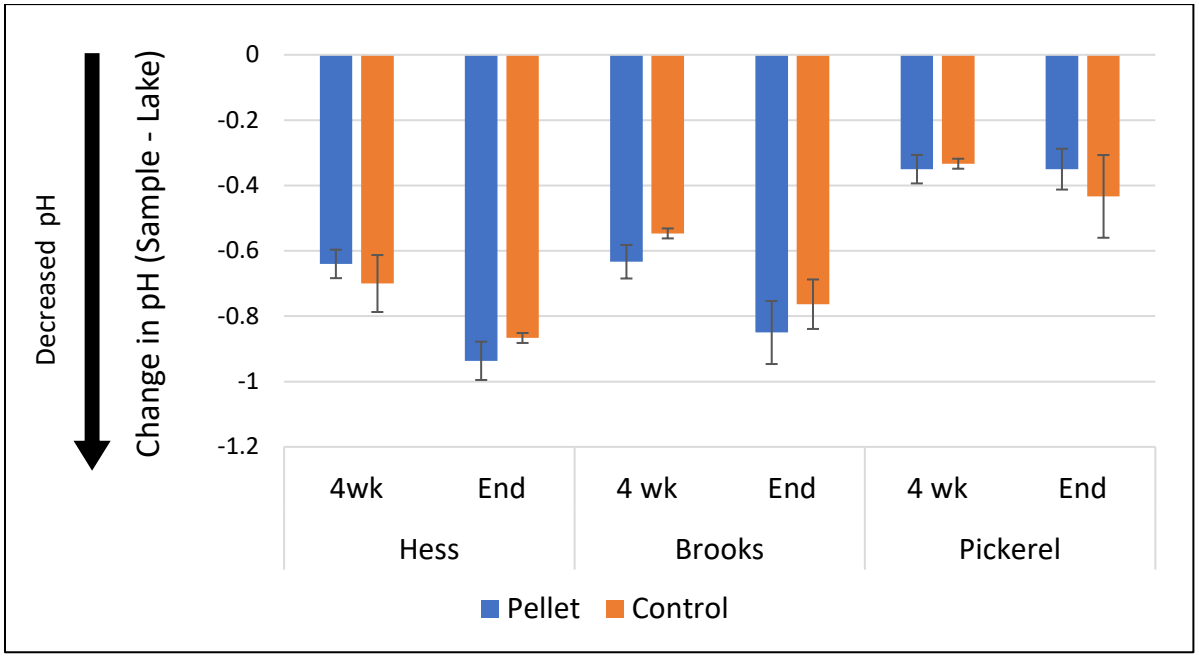

Figure 17. Changes in pH between the lake sediment and ending sediment in the bins. There were no significant differences between the changes in pelleted and control bins for any lake.

Bacterial Composition Analysis

Sequencing analysis found that *Bacillus spp*. composed ~99% of the bacteria in the Mukk Busster pellets. The addition of the muck pellets to the lake sediment samples did not appear to significantly alter the microbial community in either the tube or bin experiments (Figures 18 and 19). The dominant bacterial genera were similar across all of the samples, regardless of lake or treatment. The dominant genera included *Proteobacteria*, *Anaerolineaceae*, *Betaproteobacteria*, *Bacteroidetes*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Methylococcaceae*, and *Chloroflexi*. In the bin experiment, the *Bacillus* genera (red bar) was present in all of the muck pellet treatments (Figure 19) as would be expected, and absent in the controls. However, this trend was not as clear in the tube samples, where the presence of *Bacillus sp*. varied across all lake and treatment groups. These differences between the presence of *Bacillus sp.* in the bin and tube experiments may be due to the larger number of muck pellets used for the bin vs. the tube samples (7 pellets vs. ¼ pellet), or possible contamination, despite efforts to control for the amount of pellet per volume of sediment and avoid any cross-contamination.

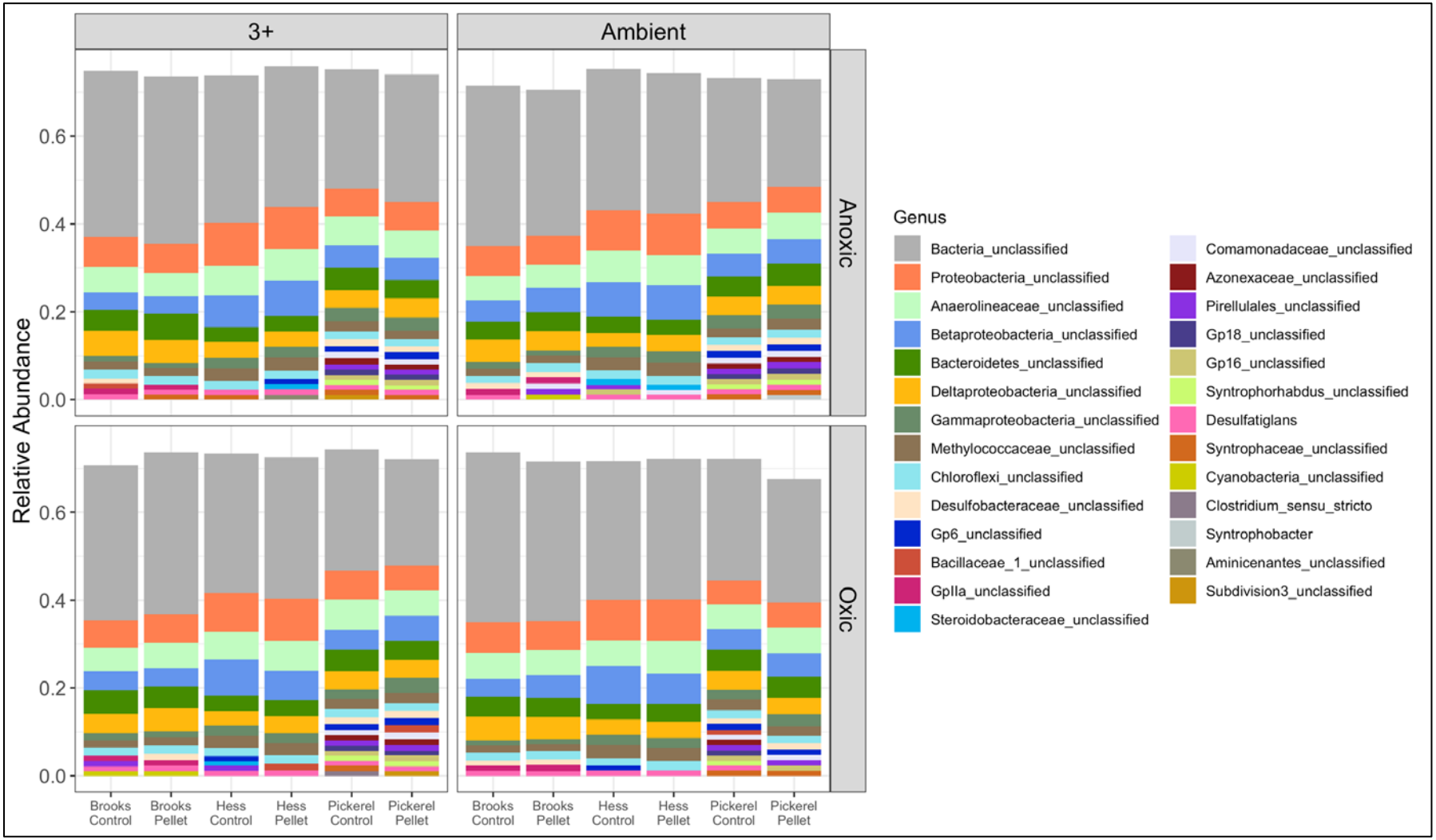

Figure 18. Relative abundance of bacterial genus-level OTUs from samples associated with the tube experiment, which evaluated the effect of pellet treatment, temperature, and dissolved oxygen on lake sediment. These include only bacterial genera composing at least 1% of the total abundance, thus the relative abundance values do not reach a total sum of 100%.

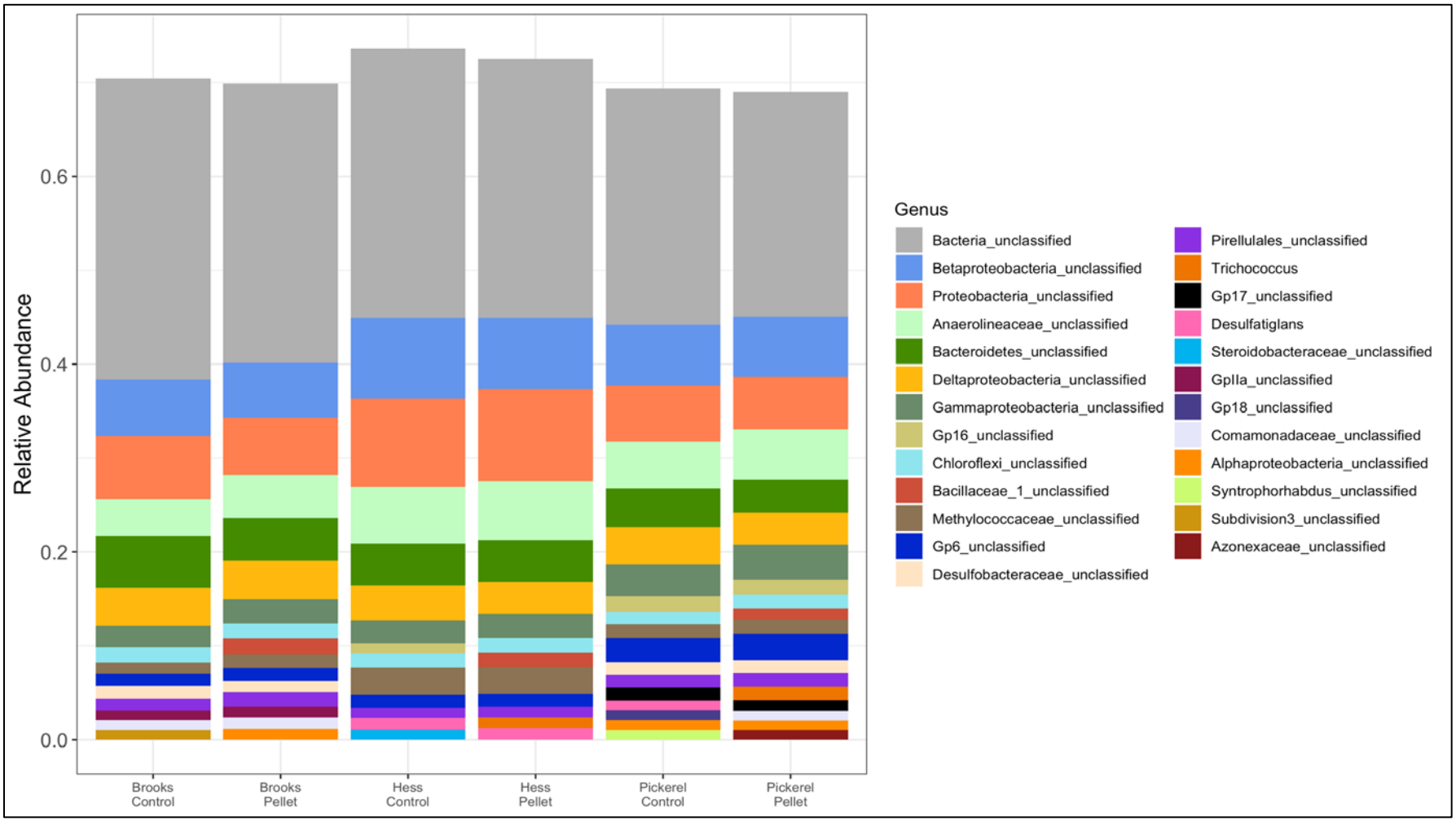

Figure 19. Relative abundance of bacterial genus-level OTUs from control and muck pellet treatments associated with the bin experiment. These include only bacterial genera composing at least 1% of the total abundance, thus the relative abundance values do not reach a total sum of 100%. *Bacillaceae* represents the bacteria introduced via the pellets.

PCoA analysis show that the majority of the variation in bacterial communities for both the tube and bin experiments is being driven by differences among lakes. For the incubator experiment, PERMANOVA analysis found that there was no influence of muck pellet treatment, temperature, or dissolved oxygen levels on these communities (Figure 20, Table 4). However, both lake and muck pellet treatment influenced the centroid location of the PCoA plot in the bin experiments (Figure 21, Table 4). This is likely being driven by differences in the number of *Bacillus sp*. and other minor bacterial groups between the control and pellet groups.

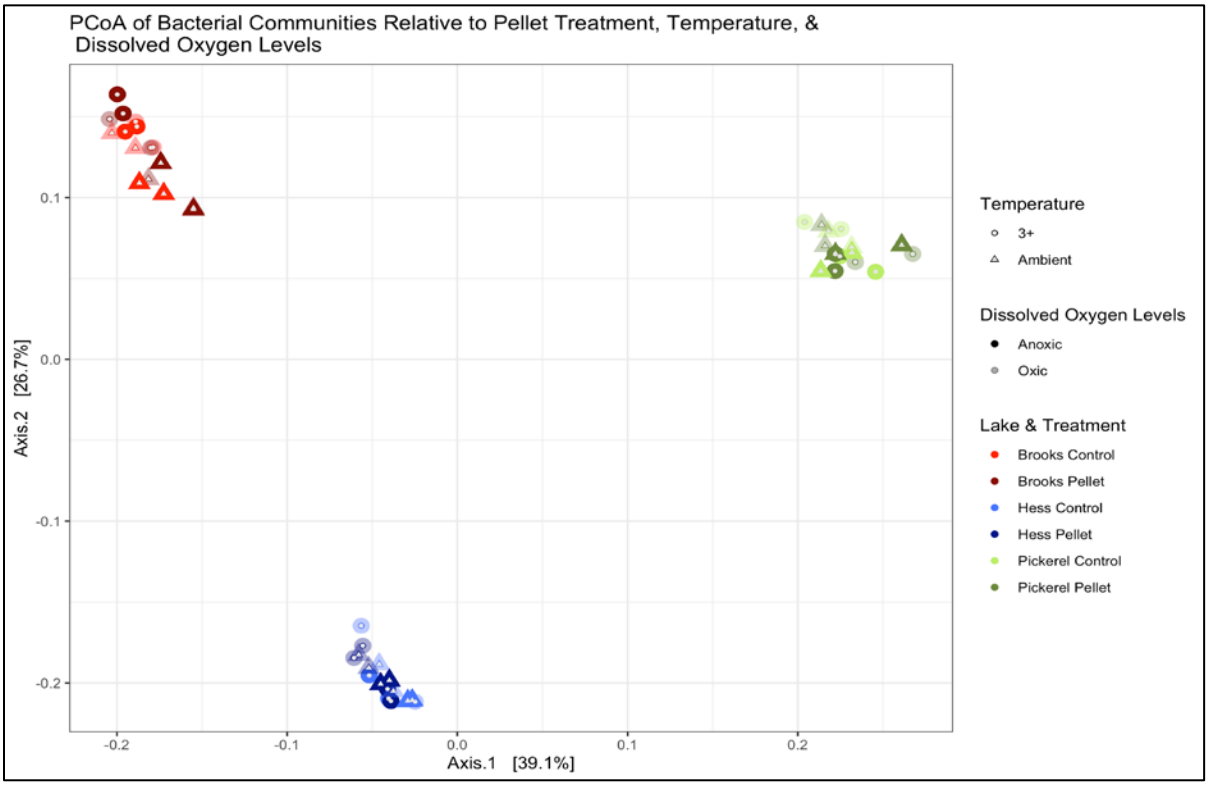

Figure 20. Principal Coordinates Analysis (PCoA) of bacterial communities associated with tube experimental samples. Differences in lake bacterial communities primarily explained the majority of the variation observed among groups.

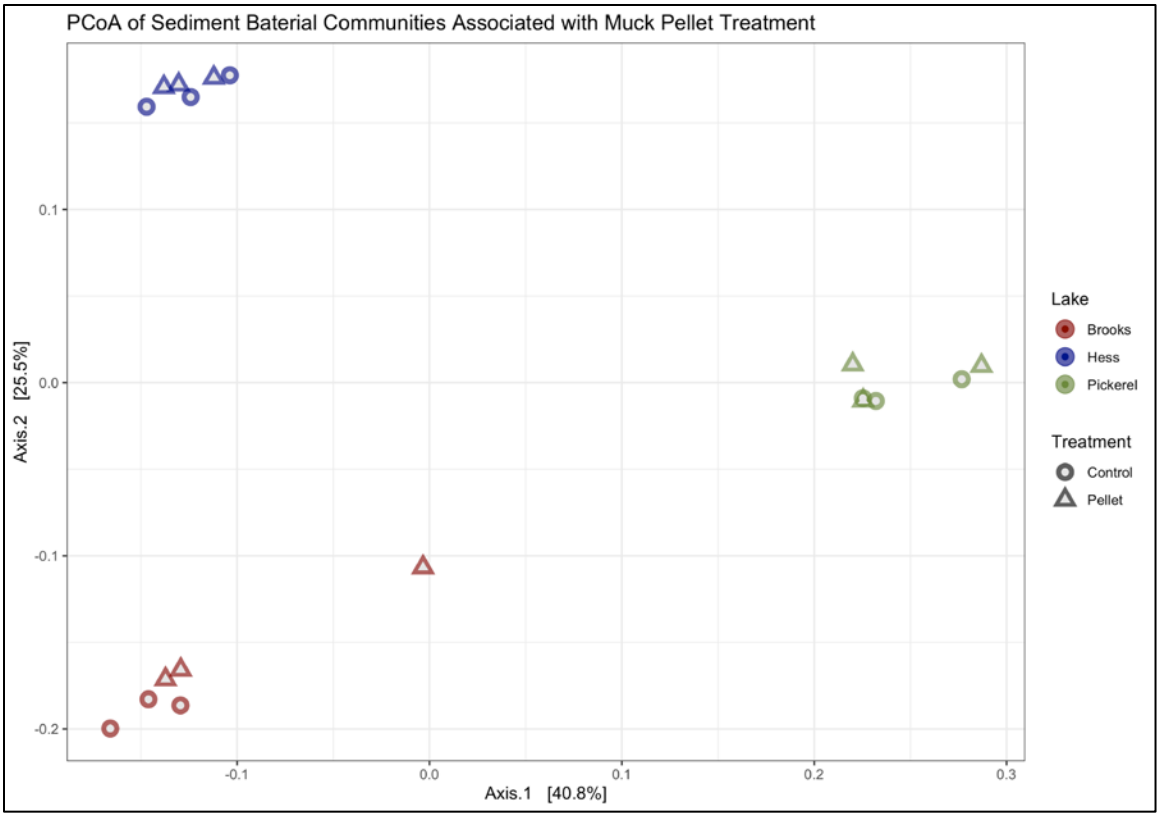

Figure 21. Principal Coordinates Analysis (PCoA) of bacterial communities associated with bin experimental samples. Differences in lake bacterial communities primarily explained the majority of the variation observed among groups.

Table 4. PERMANOVA results based on a Bray's distance matrix evaluating the influence of treatment group on centroid location of the PCoA plot for both the incubator and bin experiment.

Incubator Experiement					
	DF	*SS*	*F.Model*	*R2*	*P-value*
Lake	2	2.37	42.00	0.65	0.001
Treatment	1	0.02	0.83	0.006	0.468
Dissolved Oxygen	1	0.04	1.45	0.011	0.196
Temperature	1	0.03	1.23	0.009	0.255
Bin Experiment					
Lake	2	0.88	16.67	0.64	0.001
Treatment	1	0.1	3.91	0.07	0.008
Lake * Treatment	2	0.06	1.27	0.05	0.246

DF: Degrees of freedom, SS = Sum of Squares

For the tube experiment, differences among lakes had a significant effect on bacteria genus-level evenness ($p<0.0001$), but there were no differences among lakes in bacteria genus-level richness (Figure 22). This means that the relative number of bacterial genera was consistent across lakes, but some bacteria dominated more in specific lakes. This can be seen by the lower levels of bacteria evenness in Hess Lake. There was no effect of pellet treatment, temperature, dissolved oxygen levels, or the interaction among these factors on evenness or richness; however, this could be due to the relatively low number of sample replicates available for this comparison. Similar results were observed for the bin experiment. There was a significant difference among lakes in terms of the evenness of the bacterial genera ($p<0.0001$); however, no effect of lake or pellet treatment was observed on bacteria richness (Figure 23).

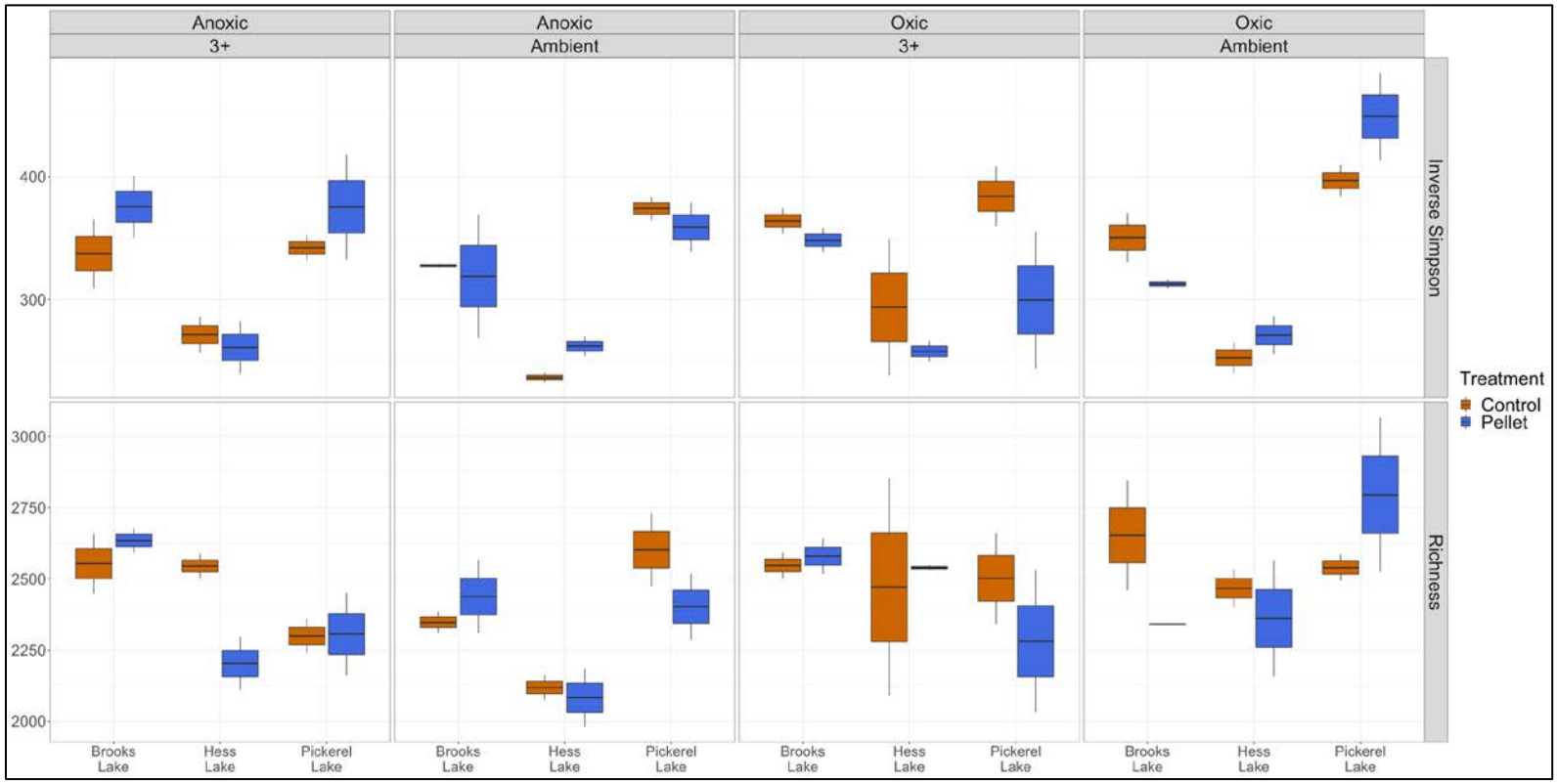

Figure 22. Bacterial genus-level alpha diversity (evenness [top panel] and richness [bottom panel]) estimates for the tube experimental samples. A significant lake effect was identified across the groups in terms of genera evenness ($p < 0.0001$). No effects of pellet treatment, temperature, dissolved oxygen, or their interactions were noted for either richness or evenness.

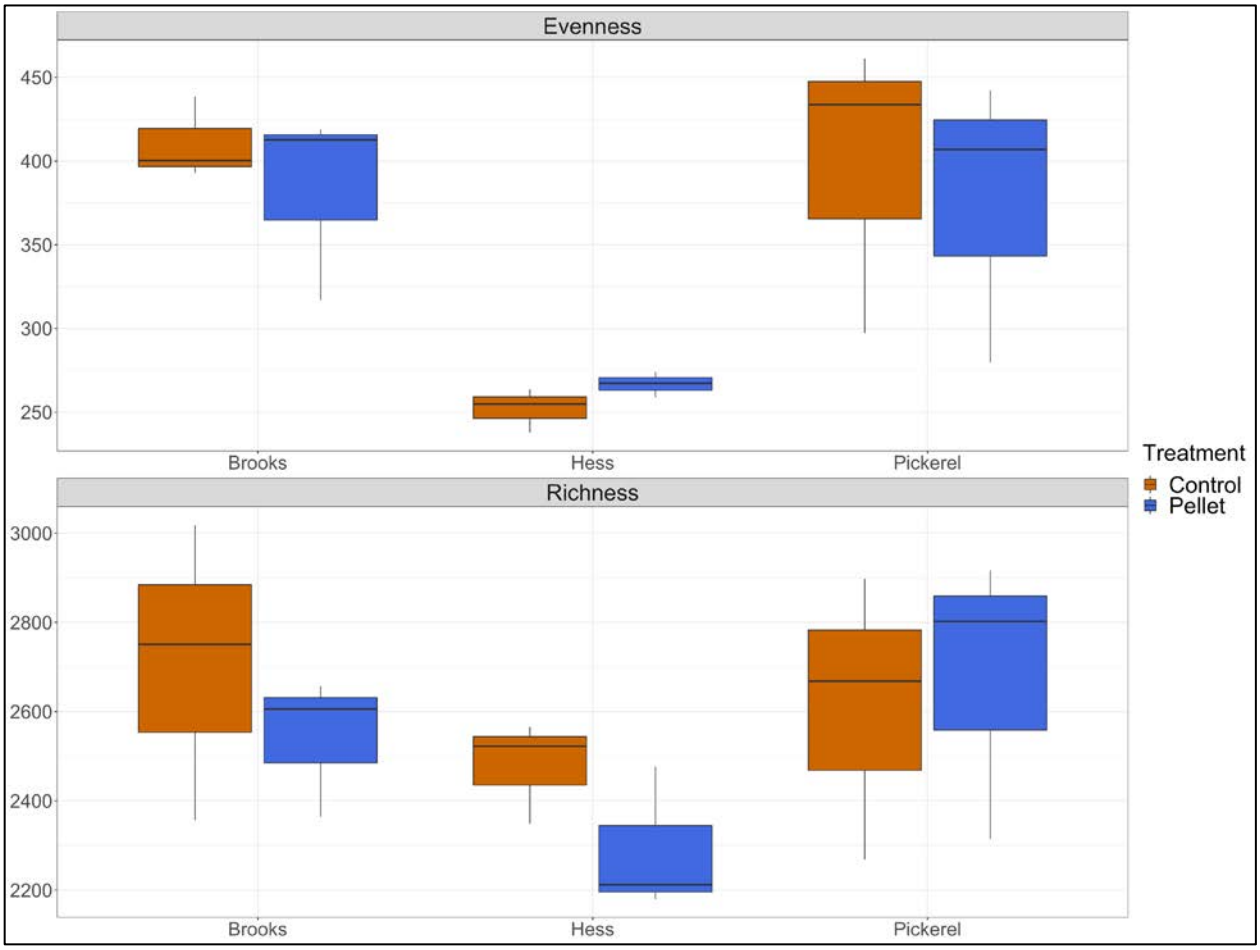

Figure 23. Bacterial genus-level alpha diversity (evenness [top panel] and richness [bottom panel]) estimates for the bin experimental samples. No significant differences in group richness were observed among lakes or treatments. A significant effect of lake was observed on bacterial evenness ($p < 0.0001$), with sediment from Hess Lake displayed significantly lower values of bacterial evenness compared to Brooks or Pickerel Lake, regardless of muck pellet treatment.

Summary

This study provides clear evidence that the application of Mukk Busster pellets do not result in a reduction of organic matter. Regardless of treatment factors and sediment volume, there were no statistically significant differences in changes of organic matter between treatments with pellets and those without pellets.

While the lack of a pellet effect was consistent among lakes and treatments, the change in organic matter did differ among lakes, with slight to no increase in Hess Lake, a significant increase in Brooks Lake, and a significant decline in Pickerel Lake. We evaluated whether the unexpected increase in Brooks Lake might have been due to algal growth in our tubes and bins. If we assume that chlorophyll accounts for 1.5% of algal AFDM (APHA et al. 1980), the increase in organic matter (~ 3 g on average) in the Brooks Lake tubes would have required chlorophyll concentrations ranging from 60 to 100 mg/L (or using the more common chl units of µg/L, a range of 60,000 to 100,000 µg/L), which clearly is not possible. We are still unable to account for this increase but we have reviewed our data files and cannot account for any errors, and because it occurred in both the tube and bin experiments, we believe the result is real.

We acknowledge several caveats in our study. First, our dosages were much higher than recommended. It is possible the resulting increased conductivity and chloride levels may have impacted bacterial community metabolism, reducing pellet effectiveness, although there was no apparent effect on bacteria community structure. Second, although the tubes and bins allowed us to control and manipulate environmental factors, they obviously do not replicate natural conditions. It is recommended, based on our findings, that pellet effectiveness be tested in natural lakes, using cylindrical tubes to isolate areas of sediment and water column, with control and treatment replicates. Finally, future studies should characterize the quality of the sediment organic matter. We measured total organic carbon, but determining to what degree the sediment is labile or refractory may also be critical.

Acknowledgements

Funding was provided through the Brooks Lake Improvement Board, the Hess Lake Improvement Board, and the Pickerel Kimball Lake Improvement Board through the Newago Drain Commissioner's office. We also acknowledge the support and planning of Erick Elgin from the Newago Co. MSU Extension Office.

We gratefully acknowledge the AWRI lab support provided by Hannah Shorkey. Brian Scull performed all phosphorus analyses in the laboratory. Syndell Parks performed the DNA extractions and prepared the sequencing libraries for the microbial analyses.

References

APHA (American Public Health Association), AWWA (American Water Works Association), and WPCF (Water Pollution Control Federation). 1980. Biological Examination: Determination of Biomass (Standing Crop). In *Standard methods: for examination of water and wastewater* (15th ed.). American Public Health Association, Washington, D.C.

Bray, J.R. and Curtis, J.T. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monogr. 27, 325-349.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N, Gilbert, J.A., Smith, G., and Knight R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME J. 8, 1621.

Lenth, RV. 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.3. https://CRAN.R-project.org/package=emmeans

Mair, P. and Wilcox, RR. (2020). Robust Statistical Methods in R Using the WRS2 Package. Behavior Research Methods, 52, 464-488.

NRCS (Natural Resources Conservation Service). 2012. Field Book for Describing and Sampling Soils (version 3.0 ed.).

McMurdie, P.J., Holmes, S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS One 8, e61217.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., and

Wagner, H. 2019. vegan: Community Ecology Package. https://CRAN.R-project.org/package=vegan.

Prieto, F.J.M. and Millero, F.J. 2002. The values of $pK_1 + pK_2$ for the dissociation of carbonic acid in seawater. Geochimica et Cosmochimica Acta 66(14): 2529-2549.

Schloss, P. D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., and Weber, C.F. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537-7541.

Slagle, Z. and Allen, M. 2016. Final Report. Evaluation of remediation projects at Lake Apopka. FWC Contract Np. 15054. University of Florida Fisheries and Aquatic Sciences.

Steinman, A.D., Lamberti, G.A., Leavitt, P., and Uzarski, D.G. 2017. Biomass and pigments of benthic algae. Pages 223-241. In: Methods in Stream Ecology. Vol 1 (3rd Ed.) R. Hauer and G. Lamberti (editors). Elsevier Press.

Sun, W.Q. 1992. Quantifying species diversity of streetside trees in our cities. Journal of Arboriculture 18, 91-93.

USEPA. 1988. Ambient water quality criteria for chloride- 1988. NTIS No. PB88-175 047.

Appendix A. Statistical significance tables for sediment ***tube*** study.

Table A1. Tube robust 3-way test results on change in percent organic matter (sample – lake).

4 week Tubes						
	Hess Lake		Brooks Lake		Pickerel Lake	
Factors	t3way	post hoc	t3way	post hoc	t3way	post hoc
temp:DO	0.240		0.038	DO only p=0.017, no sig. pairwise comparisons	0.063	
8 week Tubes						
	Hess Lake		Brooks Lake		Pickerel Lake	
Factors	t3way	post hoc	t3way	post hoc	t3way	post hoc
temp:DO	0.561		0.004	C only p=0.011 Amb < +3, AN < OX p<0.03	0.303	

Table A2. Tube robust 3-way test results on change in absolute organic matter (g, sample – lake).

4 week Tubes						
	Hess Lake		Brooks Lake		Pickerel Lake	
Factors	t3way	post hoc	t3way	post hoc	t3way	post hoc
temp:DO	0.057		0.053		0.063	P only p=0.015, no sig. pairwise comparisons
8 week Tubes						
	Hess Lake		Brooks Lake		Pickerel Lake	
Factors	t3way	post hoc	t3way	post hoc	t3way	post hoc
temp	0.024	Amb < +3 P_OX p=0.035 C_OX p=0.018	0.500		0.960	

Table A3. Tube robust 3-way test results on change in dissolved organic carbon (mg/L, sample – lake).

4 week Tubes						
	Hess Lake		Brooks Lake		Pickerel Lake	
Factors	t3way	post hoc	t3way	post hoc	t3way	post hoc
pellet	**0.022**	**PT < C Amb_OX p<0.001**	0.610		0.610	
temp	**0.041**	**Amb > +3 C_OX p=0.001**	0.870		0.450	
DO	0.181		0.608		**0.046**	**No sig. pairwise comparisons**
pellet:temp	**0.011**	**OX only p=0.009**	0.137		0.103	
temp:DO	**0.049**	**PT only p=0.021**	0.730		0.321	
8 week Tubes						
	Hess Lake		Brooks Lake		Pickerel Lake	
Factors	t3way	post hoc	t3way	post hoc	t3way	post hoc
DO	**0.001**	**AN > OX all p<0.0001**	**0.001**	**AN > OX PT_Amb, PT_+3, C_+3 p<0.004**	**0.001**	**AN > OX PT_Amb, PT_+3, C_+3 p<0.01**
temp: DO	**0.018**	**DO only for PT and C p<0.002**	0.301		0.701	

Table A4. Tube robust 3-way test results on change in dissolved CO_2 (mg/L, sample – lake).

4 week Tubes						
	Hess Lake		Brooks Lake		Pickerel Lake	
Factors	t3way	post hoc	t3way	post hoc	t3way	post hoc
temp	0.059		0.077		**0.012**	**Amb > +3 PT_AN, C_AN p<0.005**
DO	**0.001**	**AN > OX all p<0.001**	**0.001**	**AN > OX all p<0.001**	**0.001**	**AN > OX all p<0.001**
temp:DO	0.077		0.132		**0.014**	**PT only p=0.003**
8 week Tubes						
	Hess Lake		Brooks Lake		Pickerel Lake	
Factors	t3way	post hoc	t3way	post hoc	t3way	post hoc
DO	**0.001**	**AN > OX all p<0.031**	**0.001**	**AN > OX all p<0.001**	**0.001**	**AN > OX all p<0.001**

Appendix B. Statistical significance tables for sediment ***bin*** study.

Table B1. Changes in percent organic matter (sample – lake).

End Bins						
	Hess Lake		Brooks Lake		Pickerel Lake	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test	1.38 ≈ -1.94	0.132	4.90 ≈ 3.90	0.185	-2.18 ≈ -1.08	0.235

Table B2. Changes in absolute levels of organic matter (g, sample – lake).

End Bins						
	Hess Lake		Brooks Lake		Pickerel Lake	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test	1.77 ≈ -2.53	0.134	13.05 ≈ 10.74	0.291	-10.92 ≈ -5.92	0.347

Table B3. Changes in dissolved organic carbon (mg/L, sample – lake).

End Bins						
	Hess Lake		Brooks Lake		Pickerel Lake*	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test/ K-W test	4 ≈ 3	0.368	4 ≈ 3	0.368	4 ≈ 1	0.157

*if run with a t-test, the results are statistically significant: $p<0.0001$.

Table B4. Changes in dissolved CO_2 (mg/L, sample – lake).

End Bins						
	Hess Lake		Brooks Lake		Pickerel Lake	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test	3.70 ≈ 3.15	0.243	3.61 ≈ 3.10	0.435	1.72 ≈ 2.23	0.317

Table B5. Changes in total phosphorus (TP) and soluble reactive phosphorus (SRP) (both in mg/L, sample – lake).

End Bins TP						
	Hess Lake		Brooks Lake		Pickerel Lake	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test/ K-W test	**0.003 < 0.021**	**0.026**	-0.012 ≈ -0.018	0.368	**0.065 > 0.029**	**0.001**
End Bins SRP						
	Hess Lake		Brooks Lake		Pickerel Lake	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test/ K-W test	0.026 ≈ 0.039	0.077	0.004 ≈ 0.004	0.368	**0.050 > 0.020**	**0.003**

Table B6. Changes in alkalinity (meq/L, sample – lake).

End Bins						
	Hess Lake		Brooks Lake		Pickerel Lake	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test/ K-W test	50.67 ≈ 54.67	0.408	58.67 ≈ 70.00	0.130	98.00 ≈ 94.67	1.00

Table B7. Changes in specific conductance (SpCond, in µS/cm, sample - lake).

4 week Bins						
	Hess Lake		Brooks Lake		Pickerel Lake	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test	**1866 > 198**	**<0.001**	**1407 ≈ 123**	**0.051**	**1813 > 142**	**<0.001**
End Bins						
	Hess Lake		Brooks Lake		Pickerel Lake	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test	**3018 > 313**	**0.001**	**2701 > 265**	**0.006**	**3635 > 313**	**<0.001**

Table B8. Chloride for end timeframe bins.

End Bins						
	Hess Lake		Brooks Lake		Pickerel Lake	
	P vs. C means	p value	P vs. C means	p value	P vs. C means	p value
t-test	942 > 29	**0.003**	885 > 27	**< 0.001**	911 > 30	**0.015**

Table B9. Changes in pH (sample – lake).

4 week Bins						
	Hess Lake		Brooks Lake		Pickerel Lake	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test/ K-W test	-0.64 ≈ -0.7	0.366	-0.63 ≈ -0.55	0.089	-0.35 ≈ -0.33	0.585
End Bins						
	Hess Lake		Brooks Lake		Pickerel Lake	
	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value	ΔPT vs. ΔC means	p value
t-test	-0.94 ≈ -0.87	0.168	-0.85 ≈ -0.76	0.292	-0.35 ≈ -0.43	0.384