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What is the Limiting Nutrient in Winter in Urban Reservoirs? A Case Study.

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Abstract:

The importance of reservoirs in widely acknowledged by urban population, yet little is understood scientifically about their ability to process nutrients deposited into them in winter. Nutrients in waste water, lawns and construction runoff are deposited into reservoirs and several ecosystem services are lost which leads to what several researchers call "the urban syndrome". Some studies have been done on the winter limnology of lakes, yet little is understood about the same process in reservoirs. To fill this missing knowledge gap, a study on one of Nebraska's lakes (Holmes' lake) was done. In this study, we simulated how phosphorus, nitrogen and trace nutrients addition would influence this lake's phytoplankton growth in winter. We found that adding nutrients in a combination significantly increased gross primary production ($p<0.1$ in the Phosphorous+ Trace element treatment) and net primary production. In single nutrient additions we visually observed higher GPP, NPP in phosphorous and trace elements though this was not statistically significant. In this study we observed that addition of nutrients had no significant influence on extracellular respiration. These results provide ample evidence to suggest that phytoplankton activity continues in winter and Holmes' lake is nutrient co-limited.

Introduction:

Urban lakes and reservoirs provide critical ecosystem services to urban populations, yet are often at risk of degradation (Millennium Ecosystem Assessment ,2005). One of the ecosystem services that urban reservoirs and lakes support is recreation, which in turn supports physical and physiological wellbeing (Korpela et al.,2005). In fact, outdoor recreation is considered one of the most important aspects when residents are choosing a place to live (Saråu, 2015). Other benefits include provisioning (fishing) and regulating ecosystem services such as holding and processing water after major rain events. However, contrary to their need, the quality of services provided by urban reservoirs has been on continuous decline due to activities in urban areas that transfigure the natural the hydrological cycle, nutrient and soil deposition systems. This is what ecologists are currently referring to as the urban syndrome (Christopher J. Walsh et al,2005): the overall ecological loss of water quality as land uses shift. Considering waste water, runoff from construction sites, fertilizer from gardens and lawns deposited into water bodies daily, one visible effect of urban land use of water bodies is eutrophication.

Eutrophication is a result of excessive nutrient loading in lakes and reservoirs. When a previously growth-limiting nutrient like nitrogen and phosphorous becomes available, algal blooms can arise. Unfortunately, this can lead to a loss of aquatic life and recreational usability of lakes. Significant research has been done by limnologists to understand the effect nutrient over load on lake chemical, physical and biological properties. For example, the consensus is that nitrogen is the limiting nutrient in coastal water bodies and that in neutral fresh water/ in-land lakes often phosphorous is the limiting nutrient. (Ryther JH & Dunstan WM.,1971, R.E. Hecky & P. Kilham., 1988). However, more recent work

suggests that Nitrogen and Phosphorous are equally limiting in land water bodies and some of these studies indicate presence of co-limitation. (B. Moss et al.,2012, S. Muller & S. Mitrovic., 2014).

Lakes not only differ from reservoirs in the fact that they are anthropologically constructed but they also differ with respect to nutrient deposition rates, overall extent of eutrophication (Hayes, N. M et al., 2017). The first difference is that reservoirs are predicted to hold 7 times more natural river water than lakes and hold between one to three billion metric tons of sediment deposits every year (Vörösmarty et al. 1997 & Syvitski et al. 2005). Secondly, reservoirs have larger catchment areas and higher soil and particle input. Hence, they tend to have an overall lower water residence time. Lower residence time can be negatively correlated to lower processing of nutrients such as nitrogen and phosphorous (Brooks et al. (2014). Shallow depths of reservoirs provide limited nutrient deposition and processing. Considering limited nutrient deposition and lower water residence time, reservoirs are more prone to eutrophication (Hayes, N. M et al., 2017). The last notable difference is that reservoirs are more numerous and occupy larger surface area in the United states compared to lakes yet much needs to be understood about the ecological benefits associated with them and their relationship to nutrient processing and seasonal change. Therefore, considering reservoir prevalence, sediment holding and recycling capacity, it is very important to understand their response to seasonal changes and nutrient influxes.

Some work has been done to understand lake limnology during winter as indicative of spring performance (Hampton. S et al.,2017, Lars Bengtsson & Osama Ali-Maher.,2020, S. M. Powers et al 2017). So far there is very little research that has been dedicated to understanding wintering in reservoirs as an equally important part of global water ecosystem. This study aims at understanding reservoir nutrient usage and recycling in winter as a critical indicator of the usability of the reservoir in spring and summer. To assess cycling we compared growth and metabolism of phytoplankton from an urban lake in Lincoln, Nebraska, Holmes Lake in response to additions of different nutrients. Nutrient treatments consisted of nitrogen, phosphorus, and/or a cocktail of trace elements. Phytoplankton growth and metabolism was assessed using 1) temporal changes in dissolved oxygen and 2) final concentrations of chlorophyll a. Combining the chl-a approach and non-destructive dissolved oxygen sensing methods, we hope to increase our understanding of reservoir

Materials and Methods:

Site description:

The study was conducted on Holmes' lake. It is an urban reservoir located in Lincoln Nebraska (40° 46' 59.4588" N 96° 38' 11.0076" W). This 112- acre reservoir was built by the US Army Corps as 100-year-old flood control. The lake has 14 km^2 of drainage area and most of its water comes from runoff from residential areas and some from agricultural fields. This provides

continuous influx of nutrients from excess fertilizer and construction sites (Watershed Management, 2005). Currently, it is mainly used by local residents for recreational fishing and boating.

Previously this urban reservoir's water quality has fell below the EPA water quality standard (< 5.0 mg/l dissolved Oxygen) and chlorophyll-*a* concentration exceeding 0.04 mg/l (EPA, 2008). Holmes' lake has also been under rehabilitation with the most recent in 2003.

Sample collection:

Water and phytoplankton sampling were carried out at the beginning of winter (November) and mid-winter (early February). Lake turbidity was measured by lowering a Secchi disk and observing the point to which the disk ceased to be visible.

To collect lake water and phytoplankton samples, we stood over the biker's bridge across the lake and lowered a Van Dorn water sampler at least 0.2 m below the water surface. Lake water was filtered on spot using the 53 µm sieve to remove zooplankton that would hinder phytoplankton growth later. To assess water quality, pH, temperature, dissolved oxygen, and specific conductivity were measured using a handheld YSI multiprobe (Yellow Spring Instruments Inc). In February, when the lake was frozen, lake water and phytoplankton samples were collected from auger-drilled fishermen.

Chemistry analysis:

Lake water samples were analyzed for total dissolved nitrogen (TDN), dissolved organic carbon (DOC), soluble reactive phosphorous (SRP) and total phosphorous (TP). Water samples for TDN & DOC were preserved by adding concentrated HCl until 2% HCl sample concentration, frozen and later to be analyzed using the Shimadzu chemical analyzer. In the laboratory, total suspended solids (TSS) were measured using pre-weighed 1.5µm filters Whatman filters. TSS filters were later oven dried for 4 hours at 550º C and left to cool in a desiccation chamber. Chlorophyll *a* concentration, a proxy of phytoplankton biomass, was measured by filtering lake water (0.45 um Whatman filters), extracting filters in 10% magnesium carbonate-buffered methanol, and reading absorbance on the spectrophotometer (EPA Method 445.0).

Experiment:

To determine nutrient limitation of phytoplankton growth in Holmes Lake, I used a bioassay approach. Before the main experiment, a pretrial was done to assess the best methodology. Lake water was collected in October and incubated. Dissolved oxygen was measured for 6 days. From this pretrial assessment, 125 ml glass bottles were chosen for least variability among replicates and possibly the highest light penetration. In this pre-trial, after 4 days of incubation, dissolved oxygen significantly decreased which suggested that phytoplankton growth slowed after four days hence carrying out the experiment only in 4 days.

To assess the limiting nutrients in Holmes' lake at each measuring time (November and February), nitrogen, phosphorus, and a trace element cocktail were used in full factorial for the treatments. Additionally, I used a control treatment with no added nutrients. For every treatment or control, there were 4 replicates. The recipe for the trace element cocktail contained Fe, Cu, Zn and EDTA based on recipes by Kilham et al., 1998.

We used previous 2014-2017 Nebraska Department of Environment and energy data (DEE data) to determine the likely concentration of TN and TP in the water, then doubled that value for TP and added N in a 16:1 molar ratio. We doubled to reach the Red field Ratio to improve the chance that nutrients are being added proportionately to their need. (Nutrient calculations and recipe in supplemental data).

Phytoplankton growth measurement:

Within 2 hours of collection, lake water was placed in the 125 mL glass bottles and spiked with the corresponding nutrient samples. The bottles were incubated in 12-12 hours LED light and dark cycle incubator to simulate natural day and night cycles. Phytoplankton growth was assessed based on dissolved oxygen concentrations which were measured non-invasively using an oxygen sensor Pre Sens method. Measurements were done every four hours to understand plant growth across 12-day light hours for all four days. After four days, the samples were left to grow for more three days before harvest mainly due to personnel availability. Chlorophyll-*a* samples were collected by passing the whole 125ml bottle through 0.45 µm filters after incubation to understand overall biomass growth compared to the chl-a samples taken pre-nutrients addition.

From collected dissolved oxygen data respiration rate (ER), net primary production (NPP) and gross primary production (GPP) were calculated as stated by P. Staehr et al., 2010. Data analysis was done by performing a three-way ANOVA (factors: N, P, TE) on each metabolism (GPP, ER, NPP). Assumptions of normality and equal variance were tested using a visual examination of the NPP plot and Levene's test respectively. If assumptions were violated and a data transformation did not work, we tested for outliers using Cook's Distance. Those observations that have a Cook's distance greater than 4 times the mean was classified as influential and removed from the model. If ANOVA found significant results, Tukey's Honestly Significant Difference (HSD) post-hoc comparison test was used to determine treatment differences. All statistics were performed in R version 3.5.2. Levene's test was performed using the "car" package, Tukey's HSD test was performed using the "multcomp" package.

Results:

Lake water characteristics:

There were some differences in lake chemical and physical properties over winter particularly dissolved oxygen and specific conductivity. Lake turbidity increased as observed by the decreasing secchi disk measurement (Table 1). Other water quality parameters such as pH,

dissolved oxygen, specific conductivity decreased from the first sampling (19 November 2019) to the second sampling (02 February 2020).

	November	*February*
Secchi disk measurement (cm)	50	45
Temperature (ºC)	3.6	2.4
pH	8.17	7.99
Dissolved oxygen in (mg/l)	27.2	7.9
Dissolved Oxygen (%)	192.3	50.5
Specific conductivity (uS/cm2)	462.1	122.6

Table 1: Water characteristics in winter. The pH and specific conductivity, dissolved oxygen decreased in winter.

Limiting nutrients in Holmes' lake:

November:

Gross primary production (GPP):

Only trace elements in all single nutrient treatments (N, P and TE) simulated gross primary production across the duration of the experiment ($p<0.05$ at a significance level of 0.1). In treatments with combined nutrients (N+TE, N+P, P+TE) significant simulation on gross primary production was observed in all of them (fig 1). Treatments containing all nutrients (NPTE), no simulation on GPP was observed.

Net primary production (NPP):

No significant response on NPP was observed in any single nutrient treatments N, P and TE (Fig 2). Significant nutrient phytoplankton interaction (NPP) was observed in combined nutrient treatments with the highest in nitrogen+ trace element (NTE) treatments. No simulation on net primary production was observed in treatments with all nutrients (NPTE). Only third day data (fig 1&2) were plotted due to benefits of a stabilized system after the adjustment in the first two days. Statistical analysis of GPP, NPP is based on 4 days of data for all treatments and all days' data analysis are table 2.

Respiration rates: There was no significant influence of any treatment on respiration rates hence we did not report the data.

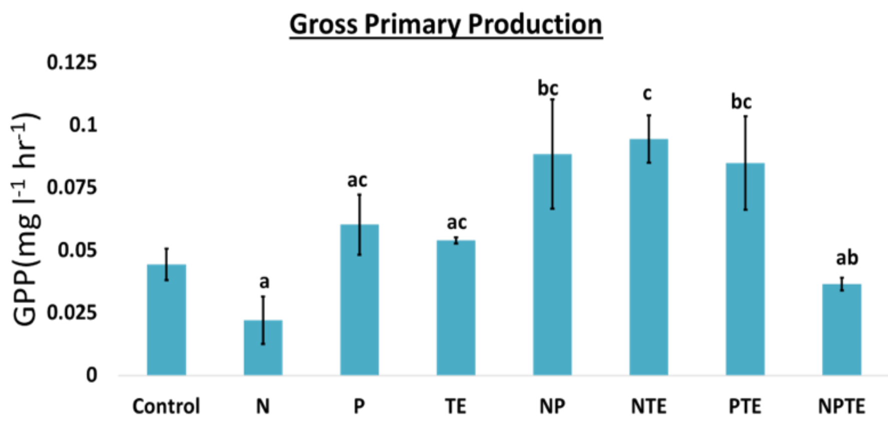

Figure1: Third day GPP simulation to nutrient addition in Holmes' lake water. The third day was chosen for graphing as the system had overall stabilized but significance was added based on analysis of the total duration of the experiment.

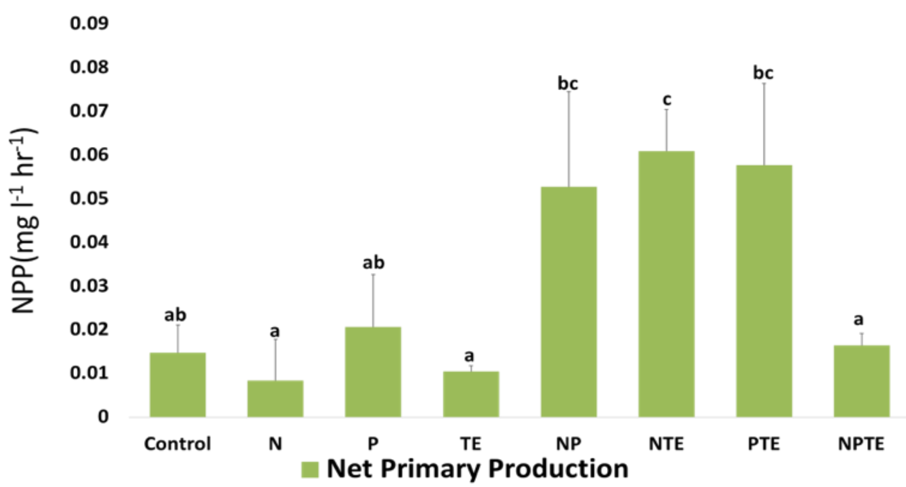

Figure2: Third day NPP simulation to nutrient addition Holmes' lake water. The third day was chosen for graphing as the system had overall stabilized but significance was added based on analysis of the total duration of the treatment.

GROSS PRIMARY PRODUCTION					
Treatments	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**(Pr>f)**
N	1	0.0000716	0.0000716	0.1400	0.7121978
P	1	0.0009970	0.0009970	1.9485	0.1780570
TE	1	0.0023040	0.0023040	4.5030	0.0465169
N+P	1	0.0010463	0.0010463	2.0450	0.1681486
N+TE	1	0.0000241	0.0000241	0.0471	0.8303509
P+TE	1	0.0066705	0.0066705	13.0369	0.0017446
N+P+ TE	1	1 0.0093110	0.0093110	18.1975	0.0003778
NET PRIMARY PRODUCTION					
N	1	0.0006915	0.0006915	2.5735	0.12434
P	1	0.0005078	0.0005078	1.8898	0.18443
TE	1	0.0001277	0.0001277	0.4752	0.49852
N+P	1	0.0009890	0.0009890	3.6807	0.06943
N+TE	1	0.0004158	0.0004158	1.5474	0.22790
P+TE	1	0.0012924	0.0012924	4.8097	0.04029
N+P+ TE	1	0.0091484	0.0091484	34.0469	1.042e-05

Table 2: GPP and NPP in November full trial after performing ANOVA in r. P-value=0.1. Significant response was observed in TE for GPP and in N+TE, N+P, P+TE for net primary production.

February:

There were at least 23 outliers identified by on Cook's distance (Fig.4). Those were removed and the data was rerun.

Gross primary production (GPP):

Overall, in single nutrient (P, N, TE) addition did not significantly increase overall gross primary production. Phosphorus simulated GPP(p=0.08814) but it was not statistically significant(fig.6). Contrary to the observed GPP significant simulation in November, **combined nutrient treatments** in February had no significant response.

No treatment had any simulation on net primary production.

Respiration:

There was evidence of N stimulation on respiration, but it was not statistically significant (p=0.07, p-value=0.01). In treatments combining trace elements with phosphorus, trace elements seemed to depress phosphorus' response. Trace elements seemed to also to dampen nitrogen's response to respiration.

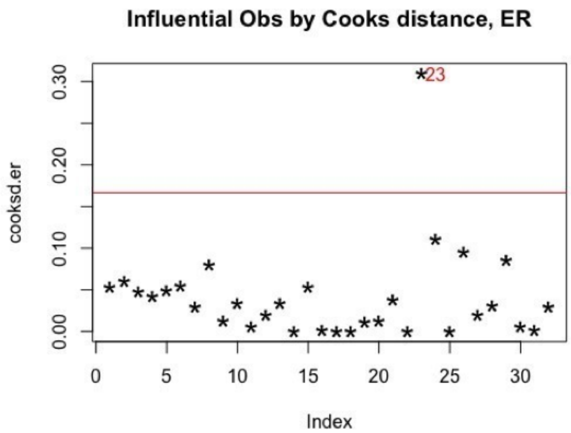

Figure 3: Cooks distance outlier response.

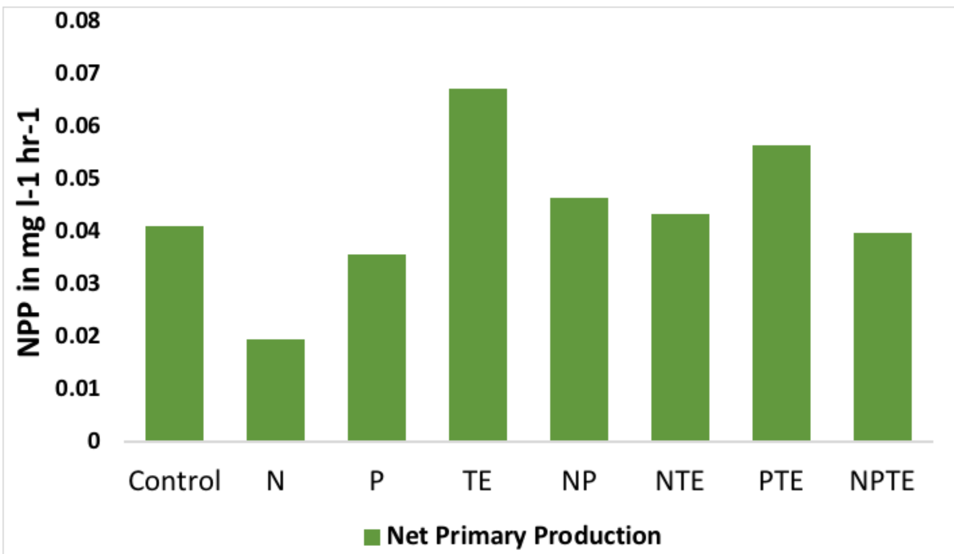

Figure4: Third day NPP simulation to nutrient addition in February. The third day was chosen for graphing as the system had overall stabilized but significance was added based on analysis of the total duration of the treatment hence no significance in all treatments.

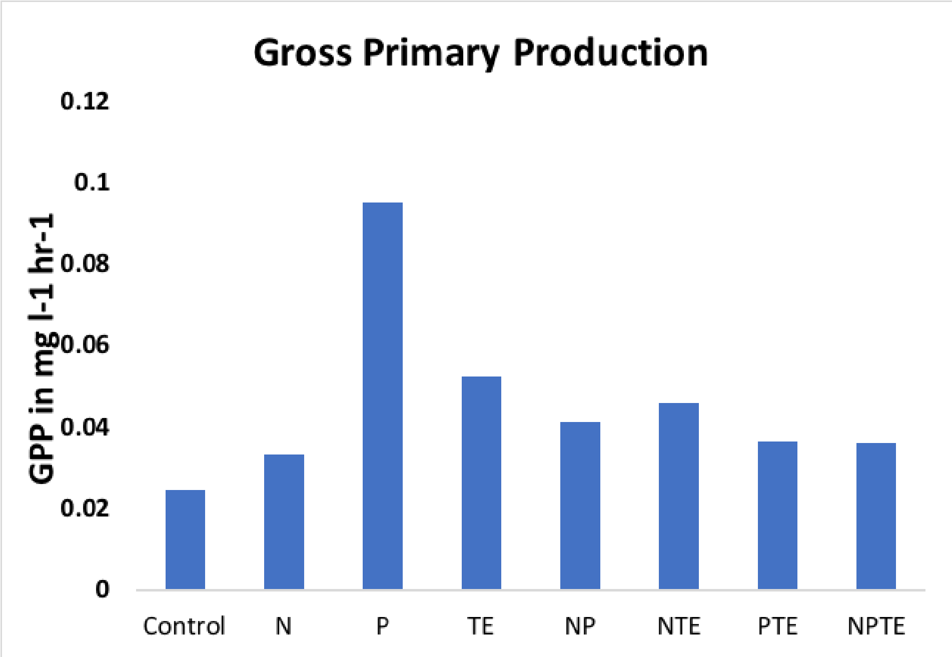

Figure5: Third day GPP in February. The third day was chosen for graphing as the system had overall stabilized but significance was added based on analysis of the total duration of the treatment.

Discussion:

Holmes' lake activity during winter:

Holmes Lake is a fairly eutrophic as the Secchi disk measurement was low and ranged between 0.4-0.6 m which is typical for eutrophic lakes (Prepas, E &Charette T.,2003). This also correlated to the total sediments (supplemental data) in the lake hence suggesting that the lack of clarity can not only attributed to the short depth of the reservoir (2-14ft) but also high sedimentation and some phytoplankton biomass in the lake.

From November 2019 to February 2020, dissolved oxygen decreased by 74% which indicates predominance of respiration over photosynthesis in winter hence an area limnologist can explore. With lowered dissolved oxygen, I still found some phytoplankton activity even in the deep of winter as I still observed phytoplankton responses to nutrient addition which would not happen if they were dead or inactive.

Nutrient limitation:

Our results provide several insights into nutrient limitation in Holmes' lake in winter. The most statistically significant ecosystem responses (GPP & NPP) were observed in combined nutrient treatments as follows.

1. Gross Primary Production was highest in PTE followed by NTE treatments. We only observed simulation to Nitrogen if it was combined with either P or TE (fig.5). This led us to believe there was nutrient co-limitation in Holmes' lake. Though this response is still novel as previous studies usually aligned with single nutrient limitation, several other studies have found co-limitation in reservoirs (Harpole et al., 2011, Romero et al.,2013, Muller & S, Mitrovic.,2014). Hence the conclusion that more than one nutrient is needed to simulate growth and biological function of the phytoplankton community in Holmes' lake.
2. Net Primary production was highest in Nitrogen and Trace element treatments (NTE) followed by PTE and NP treatments. Simulation to combined nutrient treatments with phosphorus might be due to the fact that sediments in the lake do not have enough phosphorous or it is phytoplankton unavailable hence phosphorous addition necessary to simulate significant net primary production (Conley et.,2009). On the other hand, simulation to nitrogen containing treatments indicated lower nitrogen levels in the lake and possibly cyanobacteria population in the lake do not fix enough nitrogen to simulate massive growth (Conley et.,2009). Hence necessary to complementary add both nitrogen and phosphorous to invoke ecosystem response.

Understanding co-limitation also requires understanding beyond each nutrient's role and availability but also various nutrient interactions with phytoplankton community as a whole. It possible that when nitrogen was present in combination with other nutrients such as phosphorous, the added phosphorus was used by the phytoplankton species that need both nutrients in higher amounts hence overall phytoplankton growth in combined nutrient treatments compared to when each nutrient was added separately (Wyatt et al., 2010). Nitrogen was also more responsive when combined with trace elements. We suggest this is because certain trace elements like Fe increase No3- assimilation into the phytoplankton hence increased gross primary production and net primary production when both are present (North et al.,2007).

Though trace elements and their interactions are well studied in the agronomic world, their interaction with phytoplankton is rarely understood in aquatic systems. So far only the interaction of Fe to increase N assimilation is known (North et al.,2007). Yet adding them had a strong influence on GPP and NPP of Holmes' lake which indicates a research gap that needs to be filled in the future.

<u>Respiration:</u>

It is important to note that in November nitrogen overall dampened respiration rates which lowered overall gross primary production (fig.6) This might have been to the fact that nitrogen addition can some stimulate the bloom of noxious algae hence overall low dissolved oxygen levels in nitrogen containing treatments (N. Rabalais.,2002). The observed response suggests

that nitrogen amount in the lake might more than sufficient for harmonious growth of phytoplankton and any added amount led to growth of only noxious algae (J. Elser.,1999).

The previous noted response of nitrogen's dampening of respiration in November was observed in February but this time with trace elements. Trace elements seemed to dampen respiration rates of when in combination with either nitrogen and phosphorous. There have been similar responses to trace elements but only in benthic systems (Laursen et al.2002). It is possible that addition of trace elements prohibited growth of phytoplankton population which was already low in the February cold water. The lack of competition enabled micro algae to flourish which lowered overall respiration rates in the system compared to other treatments without added trace elements.

There was no statistically significant response on either GPP, ER and NPP in February. The highest simulation was observed on NPP in phosphorous though it was not significant (fig 4). This showed as winter progresses, the phytoplankton community and biomass continuously decreased hence lower activity and response to addition of nutrients. Due to Covid-19 shutdown, we were unable to lab process chl-*a* samples and do the chemistry analysis on time. However, raw data to can be available upon request and possibly in future publications.

Conclusion:

Previously Holmes' lake has been on the impaired water's list and was removed in 2008 after a 5 million rehabilitation project. Monitoring the lake constantly and understanding gradual nutrient limitation changes is needed to maintain quality water usable by residents, the lake's ecosystem and save taxpayer $ that would be otherwise be used for major restorations after massive eutrophication and sedimentation. Our results provide understanding on urban reservoir's nutrient limitation and activity in winter. Reservoir's continue to process nutrients in winter and showed co-limitation hence management efforts of the lake should consider all nutrients in all seasons.

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Supplemental data:

Nutrient Amendment Calculations:

Bottle volume=125 ml.

Lake P= 0.13 mg/l or 4.2 µM

Target addition	16N (Red Field Ratio)	1P
µM/l	16*4.2= 67.16	4.20
Mg/l	1.08	0.13

For 0.5 ml	16.7904 mM	1.0494mM

<u>Trace elements considering 0.5 ml:</u>

Element	Volume (µM)
Fe	0.0105
Cu	1.13449E-05
Zn	0.0002269
EDTA	0.0332

<u>Salts added:</u>

<u>Salts added</u>	<u>mw</u>	<u>mg in 50 ml</u>
NaNO3	84.99	71.35
K2HPO4	174.18	9.139
ATE - FeCl	270.29	0.142
ATE - CuSO4	249.7	0.00014
ATE - ZnSO4	287.5	0.00326
ATE - EDT	372.2	0.618

<u>Total Suspended Sediments (TSS):</u>

Sample ID	Filter wt. (g)	Dry wt. (g) (post-dessic.)	Combusted wt. (g) (post-dessic.)	TSS(g) before HCl extraction
Holmes 5	0.0925	0.0958	0.1024	0.0066
Holmes 6 B	0.0945	0.0928	0.0935	0.0007
Holmes 7	0.0928	0.0932	0.0945	0.0013
Holmes 8	0.0926	0.0936	0.096	0.0024

Table 3: Due to Covid-19 related shutdown, we were unable to Hcl extract. However, we can see that suspended solids after combustion ranged between 0.0013-0.0066 g per every 75 ml of water or 6-13 g of sediments for every liter. This is from the top 0.2 m water which shows fairly high sedimentation across the lake overall.

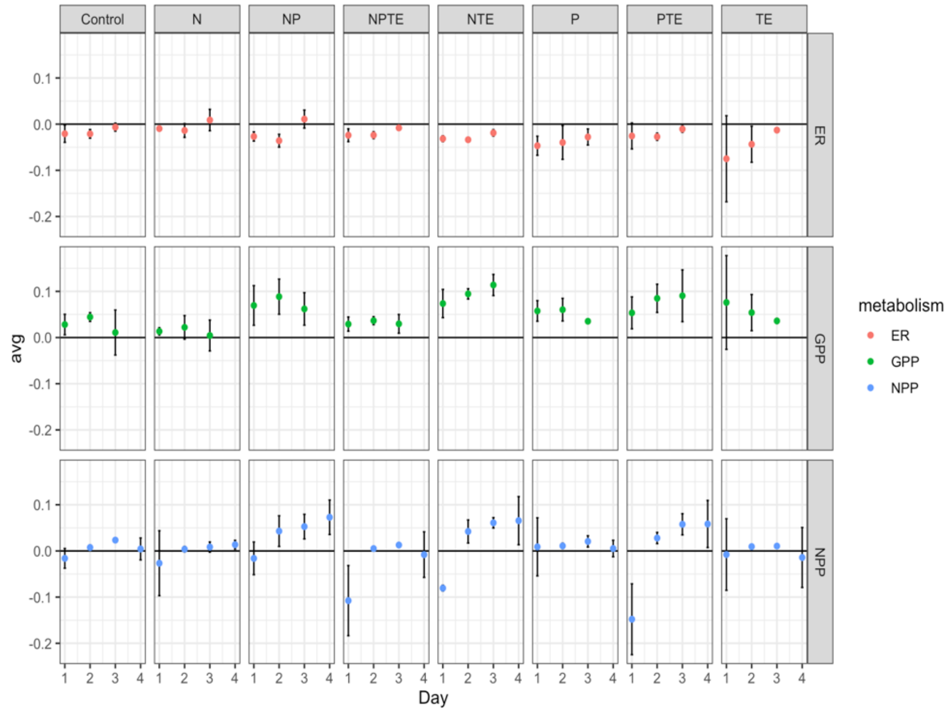

Fig 6: Means and standard deviations of metabolism by day. Treatments include Control, Single nutrient treatment such Nitrogen(N), Phosphorous (P), Trace elements (Zn, Cu, EDTA) as well as a combination of the nutrients Nitrogen and Phosphorous (NP), Phosphorous and Trace elements (PTE), Nitrogen and Trace elements (NTE) and all nutrients combined (NTE).