NUTRIENT LIMITATION AND ALGAL GROWTH ABOVE AND BELOW THE MAPLE RIVER DAM

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Abstract-

In order to determine limiting nutrient of a lotic system above and below a dam, we created nitrogen, phosphorus, nitrogen and phosphorus, and control agar bioassays. Three sites were chosen, two for both stream branches above the dam and one below. After two weeks of incubation in the river, the bioassays were removed and tested for chlorophyll a amounts. We found that the nitrogen bioassays had the greatest growth in the West branch, but every other nutrient returned the greatest growth in the Main branch. The trends show that the Main and East branches are phosphorus limited, but the West branch is nitrogen limited. This study can be used as preliminary data for future studies, after the Maple River dam is removed.

Introduction

According to Liebig's law, the nutrient that occurs in the smallest amount is the determining or limiting factor of the system. In aquatic systems, this is most often phosphorus, although the amount of nitrogen also plays a critical role (Müller et al., 2014).

Underlying geology can impact the amount of available phosphorus, as phosphorus only enters a system through weathering of sedimentary rock (Dodds, 2002). There are determining factors that influence algal ability to take up certain types of phosphorus such as pH, dissolved ions, and dissolved oxygen content, since these can dictate whether phosphorus will be soluble and able to be taken up by algae, or insoluble and unavailable to algae (Bachmann, 2014). Algal communities tend to have the greatest biomass when all else is held constant in shallow lotic systems (Taylor, 2001). Green algae also was found to have the greatest growth in conditions with 10-20 ^{o}C and irradiance of 18-175 mmol/m^{2}s (Taylor, 2001). Algae also tends to have greatest growth in slower (lower discharge) environments (Tornés et al., 2010).

Human agriculture is becoming more of a major cause of nutrient blooms, and increased algal growth in general (Paerl, 2011). Dams that have been built by humans can also impact the downstream environment. Riparian vegetation, macroinvertebrates, and the organisms that interact with them have all been shown to be impacted by dams. When the dam is removed, the ecosystem may or may not completely recover to the conditions it had before the dam was built, depending on the sensitivity of the organisms (Doyle et al., 2005).

Dam removal has been shown to shift its accompanying stream from being a phosphorus sink to a source of phosphorus (Orr et. al., 2006). Such a shift can have reverberating consequences through a system, such as changes in algal type and increases in biomass amount

(Rosemond et. al., 2000). For example, were an environment to shift to a more nitrogen poor environment after an influx of phosphorus, cyanobacteria would dominate (Sellner, 1997) and could outcompete other algae that are vital to the ecosystem structure.

The Maple River dam is set to be removed in the near future. Within other concerns about the change in river and riparian morphometry lies concern about changing the structure of nutrient limitation. In order to determine any potential future changes to downstream communities, two sites above the dam (East and West Branches) and one site below the dam (Main Branch) will be monitored (fig. 1). Differences in limiting nutrient will be determined via insertion and incubation of various different bioassays (phosphorus, nitrogen, nitrogen and phosphorus together, and a control) in order to assess the impact the future dam removal will have.

Since chlorophyll a is being used as a parameter of algae present, the East branch location is likely to have the highest initial value of algae, and grow the most algae on the phosphorus treatment, as this likely the limiting nutrient of the system. The East branch also is likely to return the greatest chlorophyll a amount on the bioassays, since it matches closest to the parameters for greatest algal growth in that it is the shallowest, slowest moving system. Phosphorus is likely to be the limiting factor as the East branch flows from a lake with significant housing around it, and likely receives nitrogen runoff from lawn fertilizer, sewage, and other anthropogenic sources. The Main branch is likely higher in algal growth, since it is the combination of the two branches before it, but will not exhibit as much growth since the nutrients from the East branch will be diluted with the water from the West branch that is not as impacted by human activities.

Materials and Methods

In order to facilitate slow release of nutrients into the water system, agar nutrient compounds were created in flower pots following the parameters set by Fairchild et al. and Tank et al. (Fairchild et al., 1985; Tank et al., 2006).

As we were to look at the differences of the three sites specifically to determine the limiting nutrient of the area, we attempted to select sites that were the closest in terms of morphometry, flow and sunlight to control for any potential confounding variables in our assessment (fig. 1). In order to measure the initial nutrients (NO_3, NH_4, PO_4, total phosphorus, total nitrogen, SiO_2, and chlorophyll a) of each site, we took water samples in acid washed bottles. To get an estimate of original levels of algae, we took chlorophyll a samples by rinsing out a plastic syringe three times at each location then filtering 120 cc of river water through a filter paper. We then wrapped the filter paper in aluminum foil and kept the paper and the water samples frozen until they could be processed by UMBS chemistry. We took initial water surface solar irradiance with a LUX photometer, and forest cover with a densiometer. We also took pH of all our nutrient sites with a Accument AP110 Portable pH meter and conductivity with a YSI conductivity meter. Densiometer readings were added for all directions and multiplied by 0.26 according to Forest Science's criterion (Lemmon, 1956).

To create our bioassays, we heated 1000 milliliters of deionized water on a hot plate and as the water began to bubble we added first agar, then the nutrient substrates. We then stirred vigorously until the solution became clear, indicating the additives had dissolved. In the nitrogen treatment 42.5 grams of $NaNO_3$ were added to result in a .5 molarity treatment. To obtain the same .5 molarity, we added 68 grams of H_2PO_4 to the phosphate treatments. We made both the individual nutrient bioassays and the control pots with 20 grams of agar per 1000 mL. We used 30 grams of agar in the treatment with both nitrogen and phosphorus substrates (42.5g and 68g, respectively), so that the solution would coagulate sufficiently.

We poured approximately 250 mL of solution into four eight centimeter tall (8.5 upper diameter) flower pots that we stopped with number ten neoprene stoppers. We placed the pots on test tube holders and allowed them to dry. After the solution had begun to gel, we placed labelled petri dish covers on top and sealed them with silicon. When the pots had adequately dried, we siliconed them onto cement blocks in sets of three, moving left to right; control, nitrogen, nitrogen and phosphorus, then phosphorus (fig. 2). To prevent nutrient mixing, we maintained distances of 5 centimeters between the stoppered part of the bioassays.

Once twenty four hours of drying time elapsed, we moved the cement blocks into the three stream locations (fig. 1); East Maple above the dam (45°34’25”N, 84°44’46”W), West Maple above the dam (45° 33’5”N, 84°47’48”W), and the main branch of the Maple River (45° 31’38”N, 84°46’27’W).

After two and a half weeks, we pulled the nutrient pots from the stream and quantified the algal growth. We removed the cement blocks from the streambeds, and covered each individual bioassay with a plastic bag. We kept the blocks out of direct sunlight as we were collecting. We scrubbed individual bioassays with a toothbrush and rinsed them with 100 mL of water. We filtered a known volume of the resulting algal slurry as described above using a chlorophyll a syringe and filter paper, and took a two mL sample of the slurry in a pipette. For chlorophyll a analysis, we stored both the pipettes and the filter papers in the freezer until chlorophyll a amount analysis at the UMBS chemistry lab.

We ran *one way ANOVA* tests to compare chlorophyll a among treatments within each site. We also used *one-way ANOVA* to compare growth of specific nutrients between the sites. We then performed *Tukey post hoc t-tests* on our data to determine significance.

Results

From our initial measure of the three nutrient sites, we found that the West branch site was the coldest, with the highest amount of both surface and underwater illumination (lux), and the highest discharge. The West branch site also had the most dissolved oxygen, and similar high levels of conductivity as the Main branch. The Main branch was the deepest of the sites, and had the greatest overhead cover of the three sites (table 1.). The West branch site had the greatest initial total nutrients (phosphorus and nitrogen) out of all the sites (table 2.). It also had the highest initial levels of chlorophyll a.

The Main branch site had the largest growth within the site on the phosphorus bioassays but it was not found statistically significant (Fig. 3., F=0.256., df=10., p=0.855., n=12.), and the highest amounts of nitrogen and phosphorus and control growth of the sites. Therefore, phosphorus is likely to be the limiting nutrient since it returned the greatest algal growth. The East branch site had the greatest increase of chlorophyll a levels on the phosphorus bioassays, and the lowest overall nitrogen change (Fig. 3., F=2.635., df=10., p=0.131., n=11.). This was not significant, but suggests that phosphorus is also limiting in the East branch. West branch showed the greatest growth on the nitrogen bioassays (Fig. 3., F=0.947., df=6., p=0.517., n=6.). From this, it appears that nitrogen may be the limiting nutrient of this site.

The West branch site also had the greatest initial chlorophyll a amounts of the sites (Table 2). Nitrogen bioassays trends were the greatest in the West branch, and the lowest in the

East (Fig. 4., F=6.527., df=5., p=0.081). The nitrogen and phosphorus treatments tended to be greatest in terms of chlorophyll a in the Main branch bioassays, and the least in the West branch (Fig. 5., F=0.462., df=6., p=0.660). The control flowerpots showed the least variance amongst all the sites, but were slightly higher in the Main branch (Fig. 6., F=0.245., df=6., p=0.793). Phosphorus treatments were fairly consistent, but slightly higher in the West branch site (Fig. 7., F=0.135., df=8., p=0.876). This, however, was not found to be statistically significant.

Discussion

Based on the trends of data, the Main and East branch sites are likely to be phosphorus limited. Since both these sites are fed from lakes that are developed and used by humans, there are likely anthropogenic sources of nitrogen in the system that make the specific sites have a higher Redfield ratio for algal growth. Specific anthropogenic point sources for nutrients could be from sewage seeping into the water supply, or from runoff of fertilizers (Schindler, 2006).

The West branch site appears to be nitrogen limited. This could be due to the fact that the West branch is the least used by humans, and therefore has the least incoming bioavailable nitrogen. Also, we lost several of the bioassays from this location, which could have severely skewed our results.

The trend of being nitrogen limited at the West branch site also could be due to the higher discharge not being conducive to nitrogen fixing cyanobacteria. Also, studies have found that mixing, nutrient levels, and temperature to a certain extent dictate the abundance of cyanobacteria within a system (Geider, 1987). It is possible that the West branch conditions were the least facilitative to cyanobacterial growth out of the sites. This however, goes against our findings that the West branch site had the highest initial total nitrogen (table 2.).

The control bioassays were highest in the Main branch site. This could indicate that the Main branch site is the most conducive to algal growth, though the initial chlorophyll a values do not reflect this. West site, with the highest initial total nitrogen and total phosphorus, has the potential to be the most productive in terms of algal growth. Since we found that this particular site appears to be nitrogen limited, it could be that the nutrients within the stream are not found in the Redfield number of 16 nitrogen to one part phosphorus (Tett et al., 1985).

The future dam removal may have some consequences, such as changing the limiting nutrient dynamics of the downstream (Main branch) site. The Main branch could also shift to a non-phosphorus limited system (Orr et. al., 2006). This shift would change the amount and type of algae (Rosemond et. al., 2000), which could subsequently change the entire river biota of the area (Scheffer et al., 2001).

Tables and Figures

Table 1. The initial parametrics for the three river sites. Locations were chosen in attempt to find the the most similar of conditions to control for confounding variables. The West branch had the highest discharge with the coldest water.

	Temperature (ºC)	Depth (cm)	Surface Irradiance (lux)	Underwater irradiance(lux)	Densiometer (% canopy cover)	Discharge (mL^3/s)	Conductivity (µS)	Dissolved Oxygen (mg/L)
East Maple	19.8	11.8	692	598	8.32	1313.1	171.4	7.2
West Maple	17.5	20.9	771	607	17.68	13796.5	335.1	8.5
Main Branch	18.5	34.4	618	602	46.02	8957.7	336.2	9.0

Table 2. *Initial nutrient data for three river sites.* The West branch of the Maple River had the highest nitrogen and total phosphorus in the water column, but the East branch had the highest bioavailable phosphorus. The West branch also showed the highest initial value of chlorophyll a, and therefore had the most abundant original algal community.

	NO_3	**NH_4**	**PO_4**	**Total P**	**Total N**	**SiO_2**	**CHL-a**
Main	165	8.7	3.23	3.09	356.4	4.43	0.699
West	254	14.2	6.51	7.12	494.4	4.93	1.352
East	6.4	8.3	8.28	5.88	301.3	3.81	0.207

Fig. 1. Locations were chosen based on similarity in morphometry, discharge, and solar irradiance. Two sites were selected above the Maple River dam (East and West branches) and one downstream of the dam (Main branch).

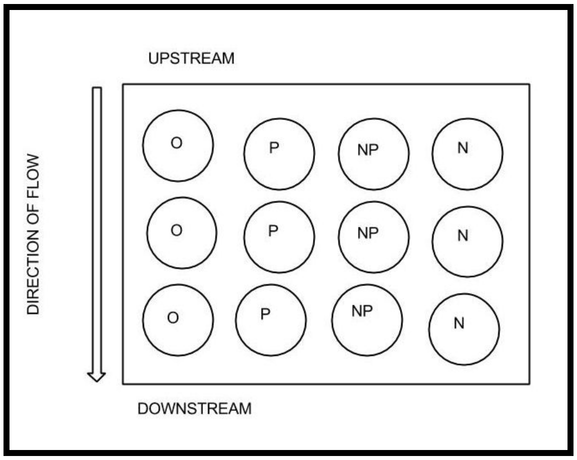

Fig. 2. *Bioassay configuration within the stream sites.* To prevent potential mixing of the nutrients, we placed the treatments side by side about five centimeters apart, with the replicate treatments downstream.

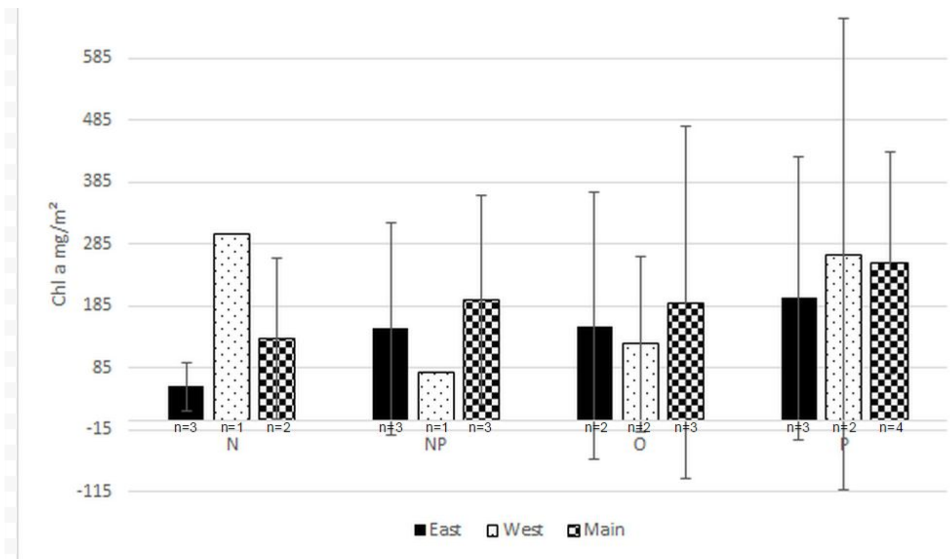

Fig 3. Chlorophyll a levels varied through each site, and by nutrient bioassay. Phosphorus tended to be a limiting nutrient in the Main Branch site. However, chlorophyll a was not significantly different among the treatments, including the control flowerpot. In the East branch, the phosphorus bioassay returned the highest chlorophyll a amounts. Nitrogen growth was the lowest at the East branch site. For the West branch, nitrogen caused the greatest change in chlorophyll a amount of this system, with phosphorus only slightly less.

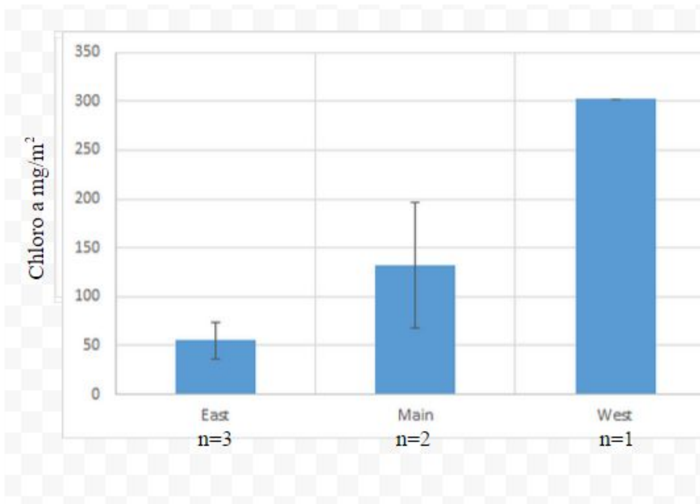

Fig. 4 Nitrogen bioassay comparison between the three sites with one standard error bar. The West Branch had the largest growth, and the East had the least. The West branch only had one bioassay remaining after the two week period, so it has no standard error.

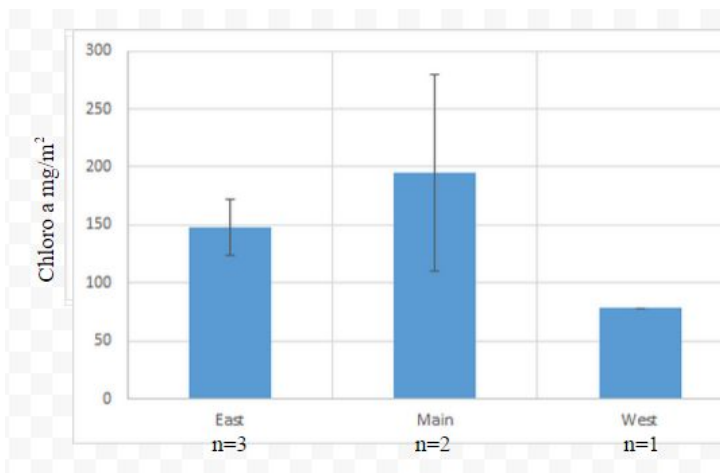

Fig. 5. Nitrogen and phosphorus bioassay comparison between the three sites with one standard error bar. The Main branch experienced the greatest growth of the three, however, the nitrogen and phosphorus treatment had the least growth of the bioassays within the Main site.

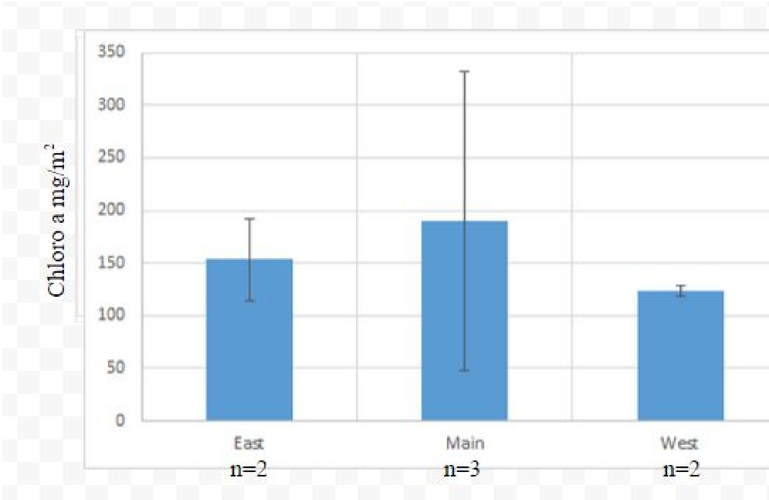

Fig. 6. Control bioassay comparison between the three sites with one standard error bar. The control pot experienced the greatest growth in the Main branch, indicating that this was the most conducive site to algal growth, even without our nutrient loading treatments.

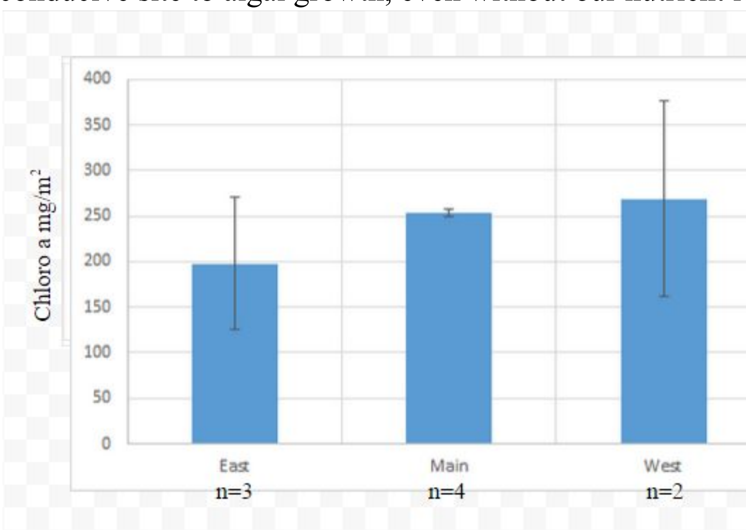

Fig. 7. Phosphorus bioassay comparison between the three sites with one standard error bar. Phosphorus was the greatest growth in both West and Main sites.

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