A comparison of nitrate and phosphate levels across three aquatic habitat types before and after leaf formation in upper Wisconsin and Michigan

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Abstract

Deciduous plants in the spring require nutrients such as nitrogen and phosphorus to build the basic materials for leaf formation. There was little information however, regarding the effects of these resource requirements on freshwater aquatic systems. I predicted that the process of leaf formation would leach nitrate and phosphate from these aquatic systems. Specifically I hypothesized that vernal ponds, which have the greatest interaction with the terrestrial environment, would exhibit the most change, while lakes, which have the least interaction with the terrestrial environment, would show the least change. I sampled three different vernal ponds, three different bogs, and three different lakes before and after deciduous plant leaf formation in the forested Upper Peninsula of Michigan. I found that nitrate and phosphate levels actually increased or did not change in vernal ponds and lakes. I did find that bog nitrate levels were significantly reduced following leaf production ($p= 0.0245, 0.0118, 0.04220$). This was probably a result of the particular hydrology of bogs. They are permanent, which means that terrestrial plants are not able to absorb water in a disproportionate amount, which would increase nutrient concentrations. They also do not exhibit a mixing in of sediment nutrients brought about by overturn, because they are not deep enough to be stratified. With this new information, this study can offer new insights on the structure of bog communities, and how these communities may show population shifts in relation to leaf formation.

Introduction

Each year, deciduous trees undergo a cycle of growing leaves in the spring, and then shedding those leaves in the fall. The production of leaves requires a large amount of resources, including carbon, water, nitrogen, and phosphorus (Jonasson and Chapin 1985). Leaf formation has been proven to have a noticeable effect on the carbon dioxide level in the air around trees (National Oceanic & Atmospheric Administration 1957-2011). Deficiencies in nutrients can lead to lower plant vitality (Yeh, et al 2000). Plants that live in bogs and other nutrient deficient habitats have become more efficient in using these nutrient resources (Aerts, et al 1999). These nutrients, may have a significant effect on the nutrient levels of lake and other freshwater aquatic ecosystems when deciduous trees are located on shorelines or in the midst of these systems.

The object of this study was to examine the influence of leaf formation on dissolved nitrogen and phosphorus in multiple aquatic systems. It has been shown that nitrogen and phosphorus have a direct effect on blue-green algae biomass (Smith 1986), so understanding the effects of deciduous trees on aquatic habitats and their nutrient levels could lead to a better understanding of the life cycles of the flora and fauna that inhabit these systems. This new knowledge could help predict how organisms boom and bust with relation to seasonal nutrient availability. It has been shown that reductions in nutrients in lakes can lead to declines in aquatic organism across all trophic levels (Wang et al. 2010). Therefore, effects of lower nitrate and phosphate levels could lead to major downward population shifts from the bottom up in trophic levels, i.e. lower nutrient levels following leaf production leads to a decrease in primary production by algae, which limits the abundance of primary consumer, which in turn leads to a reduction of secondary consumers, such as carnivorous zooplankton. In this study, nitrate and phosphate were used in lieu of total nitrogen and phosphorus, as these are the most oxidized and therefore the most usable forms, and are a good substitution for overall nitrogen and phosphorus (Sterner and Elsner 2002). The University of Notre Dame Environmental Research Center (UNDERC) east, located near Land o' Lakes Wisconsin, is suited perfectly for this study because of the large amount and diversity of aquatic habitats. The specific habitats I sampled were lakes, bogs, and vernal ponds (coordinates given in Table 1), as each type provides differing levels of interaction with the surrounding terrestrial environment.

Vernal pools have the most interaction with the terrestrial environment, because they are small shallow temporary pools with terrestrial vegetation such as trees growing in the middle of them. Bogs have the next most interaction with the terrestrial environment, but while they are also relatively small and shallow, they have no terrestrial vegetation growing in the middle of them, nor do they dry up. Lakes have the least interaction with the terrestrial environment, for they are larger and deeper than bogs, and make les contact with the surrounding shoreline per unit volume of water.

The specific hypothesis I tested is that habitats with shallow water and a greater interaction with shoreline show a greater decrease in nitrate and phosphate levels, i.e. vernal ponds with the most change, and lakes with the least, because of the different interaction levels with the surrounding terrestrial environment. I tested this by measuring phosphate and nitrate levels across multiple aquatic before and after leaf production, and projected nutrient level fluxes. In a broader sense, I also completed a general survey of the nutrient profiles of these bodies of water. Another hypothesis is that lakes will show a higher concentration of nutrients closer to the shore, because of terrestrial inputs. In addition to these two hypotheses, I asked the question of whether or not field filtering versus lab filtering water samples brought about any significant changes in nutrient levels.

Methods

Collection and Preparation

In order to test this hypothesis, three different lakes were sampled, as well as three different bogs, and three different vernal ponds (*Fig.* 1) Two rounds of sampling were done, one before leaf formation (collection dates May 16-22, 2011) and one after (collection dates June 3-4, 2011). Each site was assessed visually for interaction between deciduous plants and the water, and was rated by either having a large shoreline interaction with deciduous plants, or a small shoreline interaction with deciduous plants. Each vernal pond was sampled using one opaque Nalgene bottle, and three pseudoreplicates were derived from this sample. Each bog was sampled once with three replicates apiece by also collecting water in opaque Nalgene bottles. For lakes, three replicates were collected at shoreline, three replicates 15 meters away from the shore, and then three replicates at least 40 meters away from the shore, near the center of the lake. The samples were then strained using a 63 micron strainer and frozen for later analysis. One day before analysis, samples were thawed and filtered through Whatman 11 um filter paper using a 60 mL syringe. The same number and type of samples were taken one week after leaves were fully developed. During the second round of sampling, I also field filtered samples from the three vernal ponds, using the syringe and filter paper, for a comparison of filtering methods.

Analysis and Statistics

To test for phosphate a colorimetric analysis was used (Strickland and Parsons 1968; Wetzel and Likens 1991). Nitrate was tested using a UV Spectrophotometer (Olsen 2008). Two sample t- tests were used to compare abundance of both nutrients before and after leaf formation at each site. Two- way ANOVA's were used to determine if there were significant differences between sites of the same habitat with regard to leaf formation. My dependent variable was either nitrate or phosphate levels, and my independent variable was time of collection. Regressions were used to compare nutrient level across a gradient from shoreline to the center of all three lakes. In addition, two sample t- test were taken to compare lab versus field sampling methods.

Results

I found that phosphate and nitrate levels, as a general trend, either increased or did not change across two out of three habitats (Table 2, Table 3). The one exception to this was nitrate levels in bogs. All three bogs, Froschsee, Northgate, and Wood duck, had significantly lower nitrate levels following leaf formation ($p= 0.0245, 0.0118, 0.04220$; *Fig.* 2 a,b,c). Individual sites showed in some instances a very close relationship, such as that with phosphate levels in lakes (*Fig.* 3). No trend in nutrient level changes was observed using regression analysis of nutrient levels in lakes from shoreline to center (Table 4, Table 5). Comparing lab filtered versus field filtered samples using two sample t- tests gave inconclusive results. Two out of the six samples showed a significant difference, while the rest did not (Table 6, Table 7).

Discussion

The rest of my sites also showed some interesting patterns. Phosphate levels in vernal ponds P, K, and Red Bike exhibited a significant increase after leaf-out (p= <.0001, <.0001, <.0001; *Fig.* 3a,b,c) . With nitrate, mixed results were obtained with regard to vernal ponds. Vernal ponds P (p=<.0001; *Fig.* 4a) and Red Bike (p=0.0314; *Fig.* 4c) both showed significant increases, while vernal pond K did not (p= 0.1611; *Fig.* 4b).

One explanation for an increase of nutrients in vernal pools is based upon the nutrient needs of plants. As stated in earlier, plants require nutrients such as nitrogen and phosphorus to grow, but they also require water. Vernal pools are a ready water source for surrounding terrestrial plants (NJ Division of Fish and Wildlife 2004). Terrestrial plants could have utilized water at greater rates than nitrate or phosphate during leaf production. The pools could have been drained of more water than nutrients, thus concentrating the remaining nutrients. Another explanation could be leaf litter input into these vernal ponds. Between the two sets of collection dates, my area of study experienced a large thunderstorm. The wind from this thunderstorm added a large quantity of new leaf litter to the vernal pools I studied. While I have no actual data to support the hypothesis that chemical leaching from this added leaf litter affected the nutrient level of the vernal ponds in a positive manner, it is worth noting. Studying the effects of the addition of leaf litter and potential nutrient gains in vernal ponds could be a subject for further study.

Phosphate levels also showed a significant increase in Tenderfoot and Tuesday lakes. (p= <.0001, <.0001; *Fig.* 5a,b). Thermal mixing, which happens in late spring/early summer, releases phosphate from sediments at the lake bottom. This process has been linked to spikes in phosphorus levels (Huppert et al. 2002). This mixing is the most likely cause for the increase in phosphate levels, as my samples were taken during this period of overturn.

Nitrate levels in lakes did not vary significantly with regards to leaf formation. This could be explained by terrestrial uptake of water along with nutrients, which would reduce the noticeable impact, but more likely it is explained by in-lake nutrient cycling playing a far more important role in nutrient levels. Lakes exhibit a very tightly controlled nutrient cycling system (Likens and Bormann 1974), which does not allow for significant changes to nutrient levels, especially from terrestrial plant sources. In general lakes have very important internal drivers of nutrient production and usage (Vander Zanden et al. 2006), whose control of total nutrient levels within a lake greatly outweighs terrestrial inputs.

Nitrate levels in bogs did show a significant decrease after leaf formation. (*Fig.* 2a,b,c). This is the only data that supports my original hypothesis. It is especially interesting, for one of these sites, North Gate bog, was the only site I sampled without large deciduous trees on its shoreline (Table 8). These sites also showed no significant change in phosphate levels before and after leaf formation. This can be explained by aspects of bogs that are different from vernal ponds and lakes. Bogs, unlike vernal ponds, do not dry up, so even if water is being absorbed at a higher level than nitrate or phosphate, bogs are low enough on the water table so that the water is replaced. The nutrients however are still removed, which causes the lower concentrations. Unlike lakes, bogs are not stratified, so they do not undergo a major overturn period, which would release nutrients. My hypothesis holds, then, if a body of water is shallow enough to not undergo stratification and therefore sediment mixing, but stable enough to not dry up.

All three lakes in this study showed a similar phosphate profile ($F_{2,48}$= 1.1681, p= 0.4148; Fig 6). This is in accordance with previous study; all the lakes are all about equally low in phosphate because it tends to be the limiting nutrient for aquatic ecosystems in northern Wisconsin and the upper peninsula of Michigan (Rabalais 2002). These three lakes had significantly different nitrate profiles, and these profiles did not change with leaf formation ($F_{2,48}$=0.4488 p=0.6410; *Fig.*7) This suggests that the three lakes had vastly different internal nitrogen cycles, and that each lake was unique with regards to the amount of nitrate production.

My regression analysis did not show any significance with regards to changing nutrient levels from shoreline to lake center. One explanation for this is that these nutrients are readily soluble in water, and as such become equally distributed in the water regardless of places of nutrient input (Sterner and Elsner 2002).

Field filtering did not prove to have much effect on nitrate and phosphate levels, as only two out of the six were significantly different from one another. However, there did seem to be the pattern that field filtered samples showed much tighter grouping patterns (Table 5).With further experimentation, this could help prove that field filtering water samples is a more precise method than lab filtering. This is most likely the case because field filtering removes all plankton before freezing, while lab filtering only removes the larger of these, and the smaller microorganisms lyse when frozen releasing both nitrate and phosphate.

Nitrate and phosphate levels in vernal ponds, bogs, and lakes before and after leaf production were shown to vary widely. Some sites exhibited a reduction of nutrients, some had no change, and some showed an increase. This study above all else showed that the effect of nutrient uptake by deciduous plants for leaf production plays but a minor role in the nutrient dynamics of vernal ponds and lakes. Within the specific hydrology of bogs, however, leaf production was shown to have a significant effect on nitrate levels. Because bogs were the only systems affected by leaf formation, future studies could look exclusively at bogs to understand the influence of nutrient absorption on the communities living there. Another future study could quantify this influence by measuring water levels, uptake, and leaf compositions. A future experiment that could be done would use chemical tracers, such as nitrogen and phosphorus with stable isotope signatures, to see what percentage of aquatic nutrients is absorbed by terrestrial, deciduous plants.

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Table 1. Coordinates of the sample sites.

	N deg	**N min**	**N sec**	**W deg**	**W min**	**W sec**
Red Bike	46	13	36.97	89	31	24.48
P	46	13	40.99	89	36	36.59
K	46	14	28.47	89	28	40.49
Woodduck	46	14	29.37	89	29	7.68
Froschsee	46	14	27.00	89	30	46.74
North Gate	46	15	31.21	89	31	51.26
Tenderfoot	46	13	19.26	89	31	20.66
Roach	46	13	35.02	89	31	44.68
Tuesday	46	15	5.66	89	29	47.65

Table 2. Phosphate comparison of sites before and after leaf formation using two sample t-tests.

Site	Standard deviation, After then Before	standard error	p value	Decrease?
Vernal pond P	0.9859155	0.569218579	**1.10168E-09**	NO
	0.354452321	0.204643143		
Vernal pond K	2.254987808	1.301917818	**7.91631E-08**	NO
	1.479432044	0.854150489		
Vernal pond Red Bike	0.9859155	0.569218579	**3.04801E-05**	NO
	0.354452321	0.204643143		
Bog Froschsee	1.713454234	0.989263263	0.709929727	NO
	2.276874302	1.314553991		
Bog North Gate	6.721325886	3.88055931	0.613067777	NO
	30.90557232	17.8433405		
Bog Woodduck	0.8252768	0.476473782	0.110805779	NO
	6.842712938	3.950642157		
Tuesday Lake Shore	1.181196772	0.681964274	0.084227281	NO
	0.494631632	0.285575706		
Tuesday Lake 15m	1.632426165	0.942481686	**0.017943181**	NO
	0.293192395	0.169274708		
Tuesday Lake Center	1.063356963	0.613929429	**0.029305337**	NO
	0.825276799	0.476473782		
Roach shore	0.507824123	0.293192394	0.613882777	NO
	6.230703202	3.597298171		
Roach 15m	0.856727117	0.494631631	0.116187229	NO
	2.927409815	1.690140845		
Roach Center	0.775714161	0.44785878	0.101384159	NO
	2.00343552	1.156684037		
Tenderfoot Lake shore	0.53323083	0.307860964	**0.000553188**	NO
	0.215144399	0.124213677		
Tenderfoot Lake 15m	0.422535211	0.243950818	**0.000978089**	NO
	0.37264103	0.215144399		
Tenderfoot Lake Center	1.490564119	0.860577595	**0.017448304**	NO
	0.694772234	0.401126936		
Tuesday Whole Lake	1.175116527	0.678453843	**5.26441E-05**	NO
	0.870445832	0.502552135		
Roach Whole Lake	0.762097797	0.439997368	0.340943779	NO
	4.188282883	2.41810625		
Tenderfoot Whole Lake	1.001167517	0.578024336	**1.55401E-07**	NO
	0.500170792	0.288773742		

Table 3. Nitrate comparison of sites before and after leaf formation using two sample t-tests.

Site	Standard deviation, After then Before	standard error	p value	Decrease?
Vernal pond P	5.686759476	3.283252114	**1.05E-05**	NO
	7.600857408	4.38835707		
Vernal pond K	158.8766227	91.72746087	0.161145	NO
	15.08728714	8.710649293		
Vernal pond Red Bike	139.7301499	80.67323968	**0.031399**	NO
	0.495008417	0.285793243		
Bog Froschsee	31.46457903	18.16608317	**0.024532**	YES
	41.69037339	24.0699483		
Bog North Gate	13.07347059	7.547971766	**0.011831**	YES
	26.80547021	15.47614544		
Bog Woodduck	15.44925996	8.919634397	**0.042196**	YES
	73.06619191	42.18478557		
Tuesday Lake Shore	41.58372458	24.00837458	0.408582	NO
	29.47291355	17.01619457		
Tuesday Lake 15m	16.08830735	9.288588578	0.052361	NO
	9.040499617	5.219534888		
Tuesday Lake Center	23.29219898	13.44775735	0.108712	NO
	7.770218787	4.486137908		
Roach shore	10.54201722	6.086436478	0.464158	NO
	1.817613912	1.049399881		
Roach 15m	4.56270753	2.63428042	0.566692	NO
	2.742356044	1.5833		
Roach Center	0.005196152	0.003	0.116195	NO
	4.957731336	2.862347521		
Tenderfoot Lake shore	14.02748136	8.09877014	0.885773	NO
	32.89672527	18.99293319		
Tenderfoot Lake 15m	23.50077091	13.56817641	0.981769	NO
	6.786331852	3.918090521		
Tenderfoot Lake Center	3.695041723	2.133333333	0.213842	NO
	14.48442727	8.362587983		
Tuesday Whole Lake	38.80056764	22.40151817	0.538984	NO
	18.8049435	10.85703919		
Roach Whole Lake	6.398417187	3.694127885	0.873936	NO
	3.638064084	2.100437278		
Tenderfoot Whole Lake	14.50427992	8.374049918	0.687288	NO
	19.39060519	11.19517112		

Table 4. R-squared, standard error, and p value for six total regressions used to look at phosphate levels from shoreline to lake center in three different lakes.

Site	R squared	Standard Error	p value
Tuesday lake Before	0.120004462	0.009669392	0.361103891
Tuesday lake After	0.023752197	0.013749222	0.692182692
Roach lake Before	0.059849841	0.010484841	0.525810897
Roach lake After	8.96472E-05	0.001967517	0.980712936
Tenderfoot lake Before	0.015417802	0.001544868	0.750273615
Tenderfoot lake After	0.058611284	0.003023697	0.530260882

Table 5. R-squared, standard error, and p value for six total regressions used to look at nitrate levels from shoreline to lake center in three different lakes.

Site	R squared	Standard Error	p value
Tuesday lake Before	0.028354507	0.219504345	0.664965
Tuesday lake After	0.405521655	0.354260938	0.06514
Roach lake Before	0.328991351	0.007694167	0.106363
Roach lake After	0.15964886	0.015143637	0.286684
Tenderfoot lake Before	0.052601749	0.058749565	0.55277
Tenderfoot lake After	0.034642287	0.044359568	0.631607

Table 6. Comparisons of phosphate levels in lab filtered vernal pond samples versus field filtered vernal pond samples.

Site	Standard Deviation	Standard Error	p value
Vernal Pond P- Lab Filter	0.9859155	0.569218579	0.166132502
Vernal Pond P- Field Filter	30.47508934	17.59480104	
Vernal Pond K- Lab Filter	2.254987808	1.301917818	0.067875872
Vernal Pond K- Field filter	2.889899234	1.668484101	
Vernal Pond Red Bike- Lab	0.293192394	0.169274708	0.015274813
Vernal Pond Red Bike- Field	0.354452321	0.204643143	

Table 7. Comparisons of nitrate levels in lab filtered vernal pond samples versus field filtered vernal pond samples.

Site	Standard Deviation	Standard Error	p value
Vernal Pond P- Lab Filter	5.686759476	3.283252114	0.008508
Vernal Pond P- Field Filter	3.887934327	2.24469993	
Vernal Pond K- Lab Filter	158.8766227	91.72746087	0.446131
Vernal Pond K- Field filter	7.289042004	4.208330363	
Vernal Pond Red Bike- Lab	139.7301499	80.67323968	0.277682
Vernal Pond Red Bike- Field	10.26033788	5.923808835	

Table 8. Visual assessment of interaction with terrestrial deciduous plants.

Site	Interaction with terrestrial Deciduous plants
Vernal pond P	Large
Vernal pond K	Large
Vernal pond Red Bike	Large
Bog Froschsee	Large
Bog North Gate	Small
Bog Woodduck	Large
Tuesday lake	Large
Roach lake	Large
Tenderfoot lake	Large

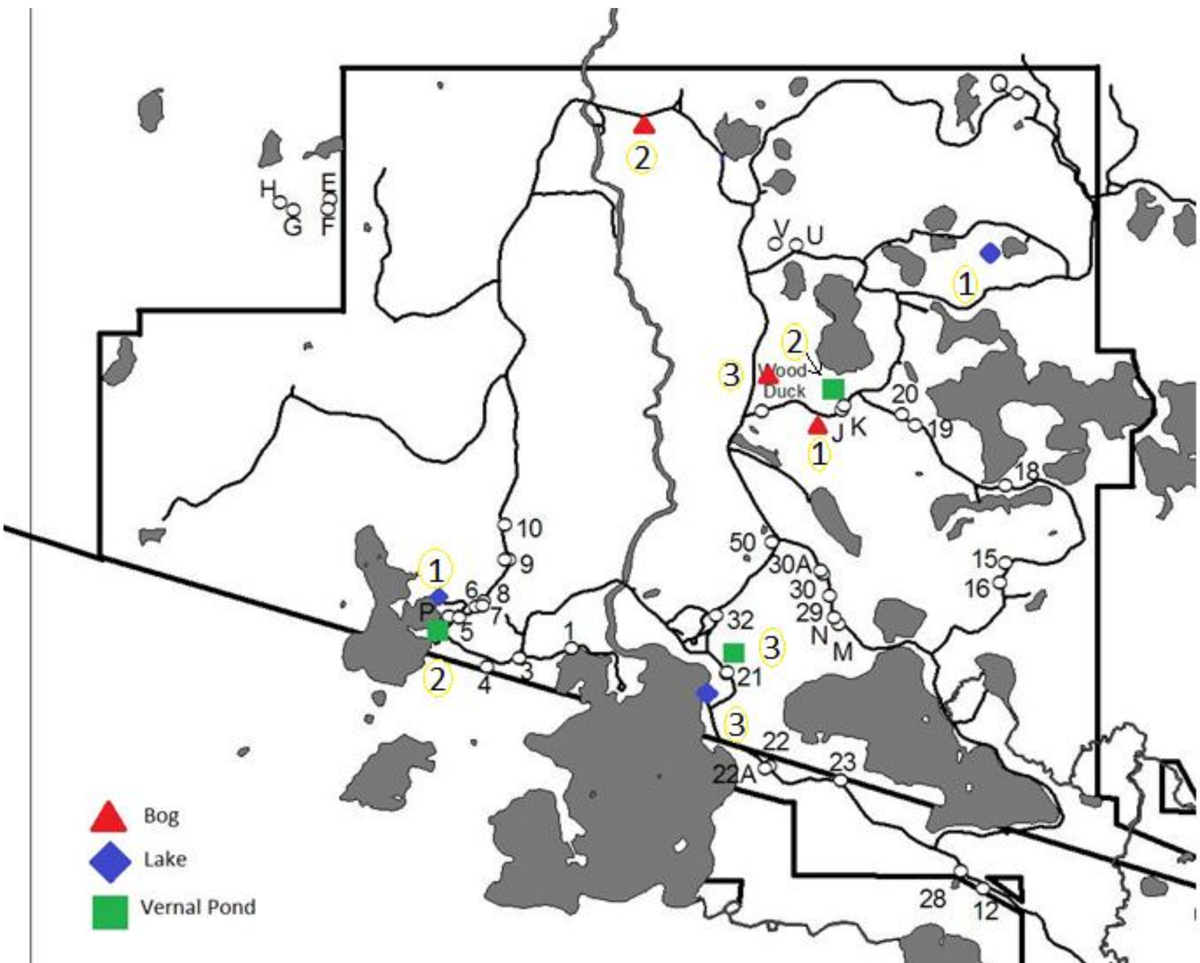


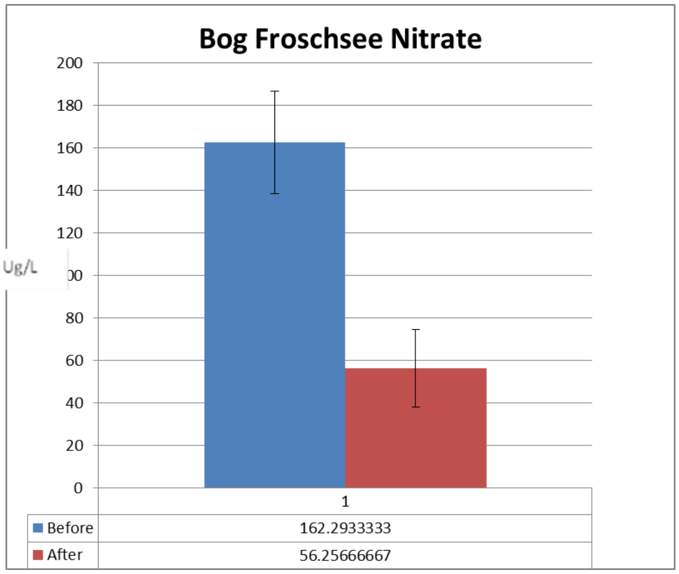
Figure 1. Map of the UNDERC property with the sample sites labeled.

Lakes: 1: Tuesday, 2: Roach, 3: Tenderfoot

Bogs: 1: Froschsee, 2: North Gate, 3: Wood Duck

Vernal ponds: 1: P, 2: K, 3: Red Bike

A.



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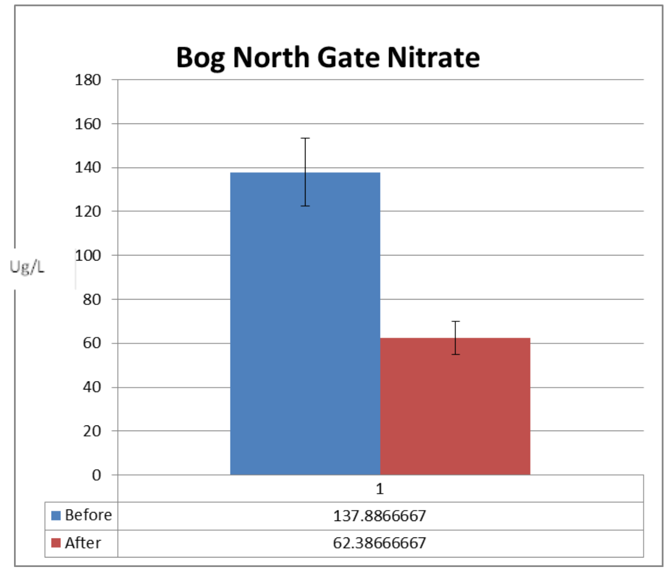

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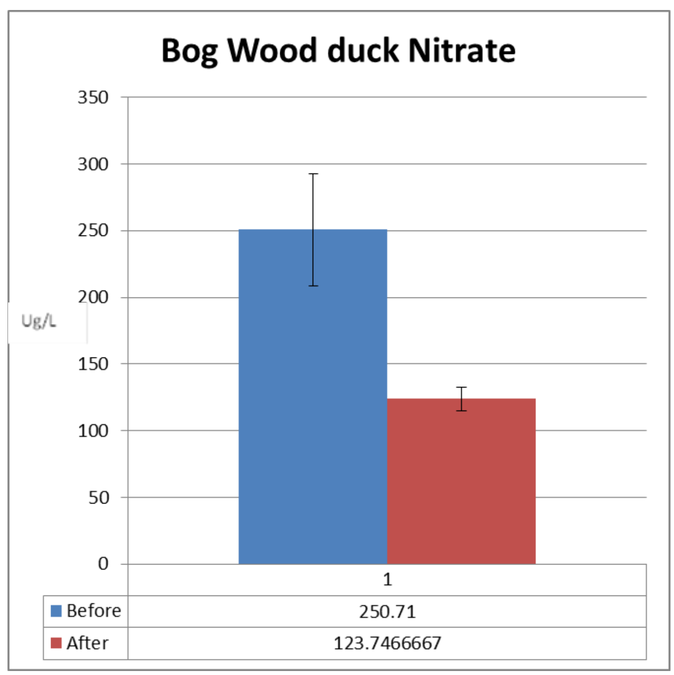


Figure 2: a, b, and c. A comparison of nitrate levels in micrograms per Liter before and after leaf formation in three different bogs. All bogs exhibited a significant decrease in nitrate ($p= 0.0245, 0.0118, 0.04220$, respectively).

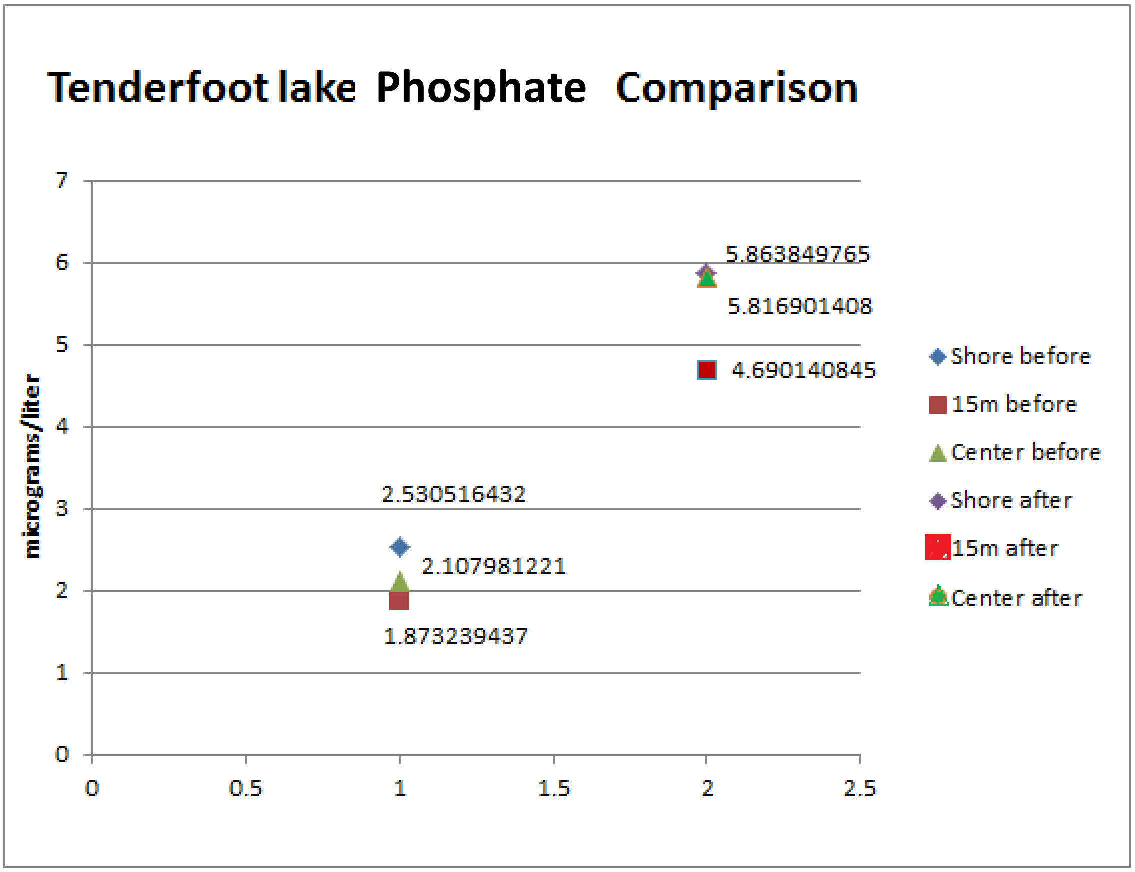
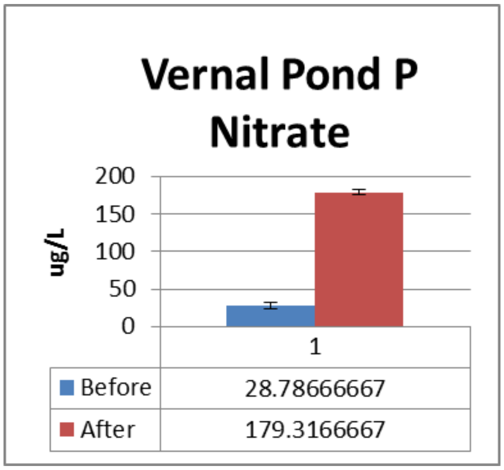
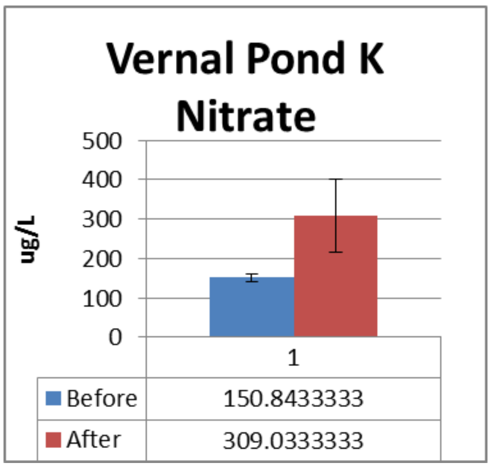


Figure 3. Comparison of phosphate levels in different samples in Tenderfoot Lake. P values too small to show on graph, refer to Table 1.





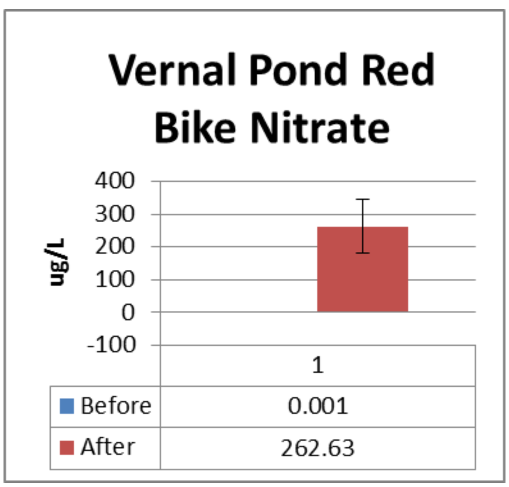
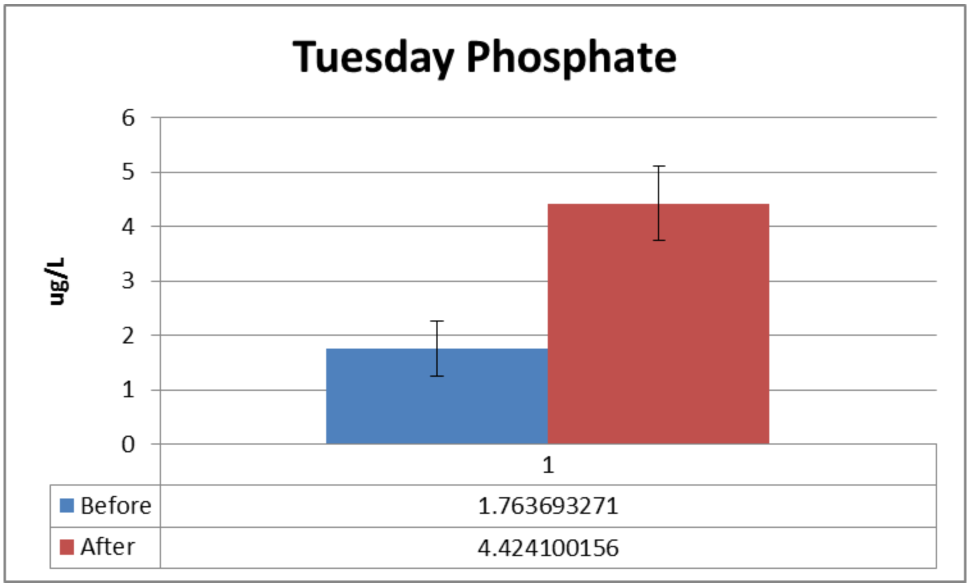


Figure 4 a,b,c. A comparison of nitrate levels before and after leaf formation in three vernal ponds. Ponds P and Red Bike increased in nitrate levels significantly p=<.0001, 0.0314). K did not (p=0.0314).



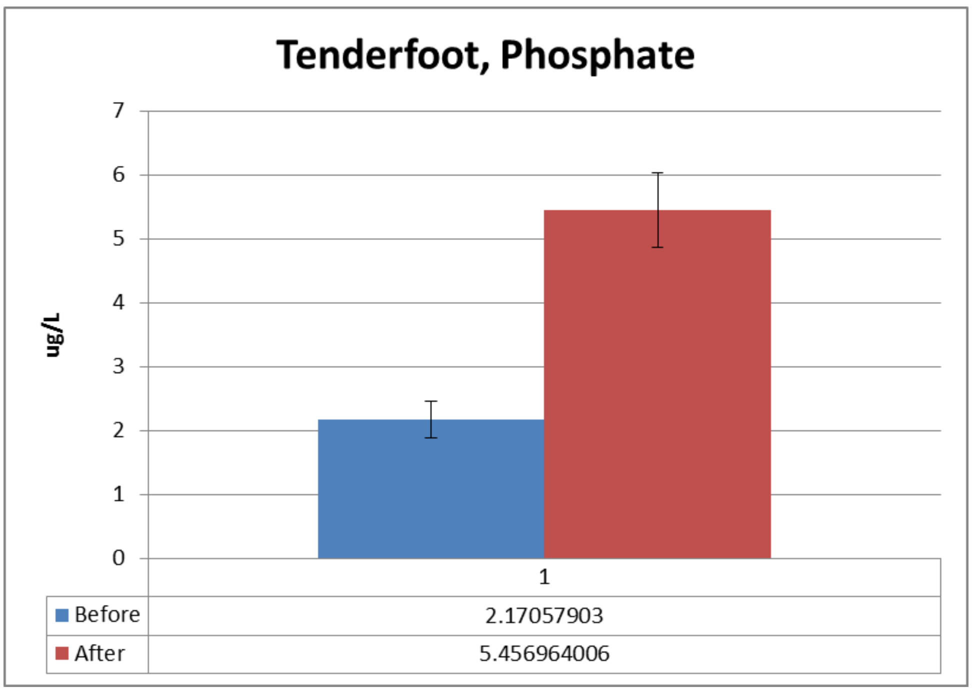


Figure 5 a, b. Comparison of Phosphate levels in Tenderfoot and Tuesday lakes. Both Lakes had a significant increase in phosphate after leaf formation (p= <.0001, <.0001)

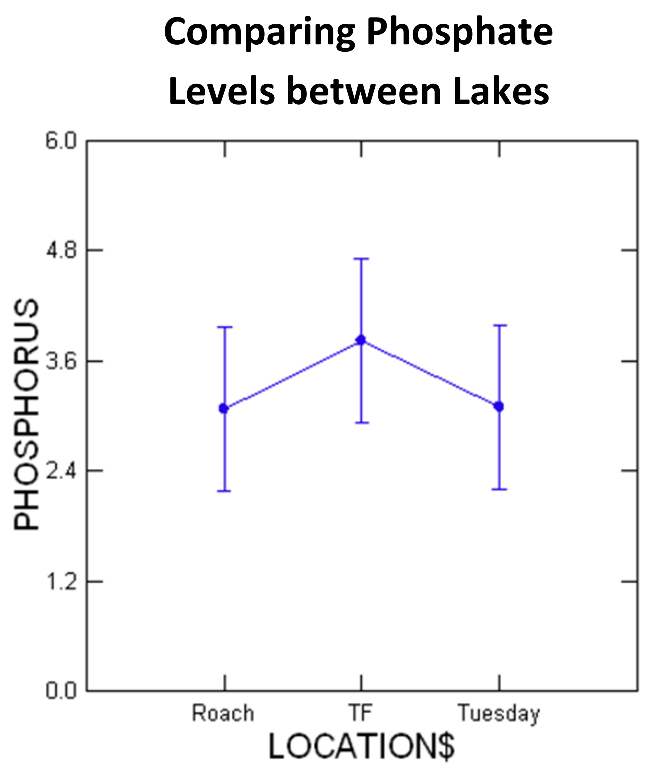


Figure 6. Comparison of the phosphate levels between all three lakes. They were not significantly different. ($F_{2,48}$= 1.1681, p= 0.4148)

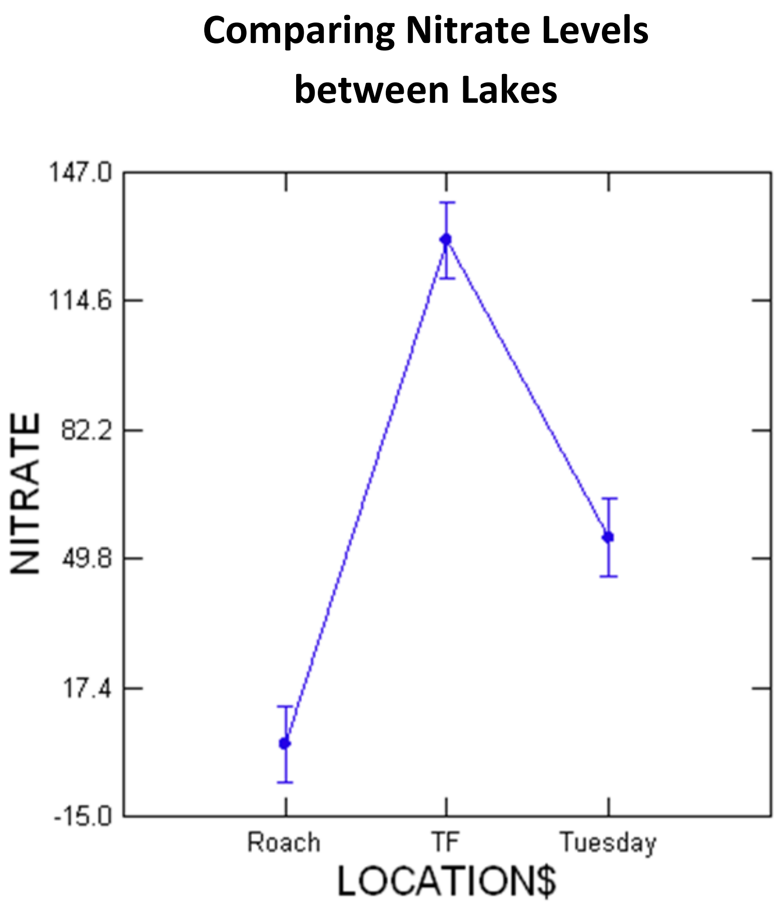


Figure 7. Comparison of the nitrate levels between all three lakes. The lakes did differ significantly ($F_{2,48}$=0.4488, p=0.6410).