NUTRIENT LIMITATION OF OKLAHOMA RESERVOIRS: POTENTIAL EFFECTS OF WATERSHED LAND COVER AND IRON

By

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Bachelor of Science in Biology

University of Central Oklahoma

Edmond, OK

2015

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2017

NUTRIENT LIMITATION OF OKLAHOMA

RESERVOIRS: POTENTIAL EFFECTS OF

WATERSHED LAND COVER AND IRON

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ACKNOWLEDGEMENTS

I would first like to thank my advisor Andy Dzialowski for all he has done for me as well as this project. His willingness to take me on as a graduate student allowed me to pursue this degree, and has inspired my passion for water quality research. His advisement and constant support has helped me grow as not only a student but also a professional. I know I would not be where I am today or able to be continuing on in this field if it were not for him, and I am forever appreciative and thankful for that. I also thank my committee members Puni Jeyasingh and Dan Storm for their support and advice on my research. Their comments have helped to improve this thesis and I am very appreciative for their time and assistance.

A big thank you to my lab/office mate Bill Mausbach, who was always willing to help and give advice when needed. He made learning the ropes of the lab much easier, as well as helping me with some statistical software and GIS, and was always a great person to chat with. I also thank the Jeyasingh lab, who were very helpful with helping me learn how to use some of their equipment as well as providing the iron for this study. Thank you to the Department of Integrative Biology for their support, as well as all the other graduate students who I have been lucky enough to meet and form friendships with. Going through this together made the stress much easier to manage.

Thank you to my parents David and Vonica, who have always been my biggest supporters from day one. Their sacrifices growing up made it to where I could experience a life where I was able to pursue my dreams without ever feeling as if that wasn't an option. I want to thank them especially for raising me in a way where I never felt there was anything I couldn't do, and always let me have full responsibility over my life and my actions. Now that independence has helped me succeed in many more ways than just one. I also thank two of the strongest women I have had the pleasure of growing up with, Jennica Dominguez and Sara Hofferber. Thank you both for the influence you have had on my life, as without your support starting years ago this would not have been possible. Thank you for always not only reminding me life is what you choose to make it, but also personally showing through your own success that it is not where you come from or what happens to you that defines your circumstances.

Finally, I thank my pets Hercules and Link (R.I.P.). Both were with me through this journey and offered constant companionship and love, regardless of how long I had to leave them home alone to be in the lab working or in the office writing.

Name: FELICIA OSBURN

Date of Degree: JULY, 2017

Title of Study: NUTRIENT LIMITATION OF OKLAHOMA RESERVOIRS: POTENTIAL EFFECTS OF WATERSHED LAND COVER AND IRON

Major Field: INTEGRATIVE BIOLOGY

Abstract: Historically, phosphorus (P) has been considered to be the limiting nutrient of primary production in freshwater ecosystems, and many efforts to control eutrophication have centered around P. However, recent research suggests that other elements including nitrogen (N) and iron (Fe), may also limit primary production. In this study, 25 Oklahoma reservoirs were selected that represented a gradient in trophic state from mesotrophic to hypereutrophic, as well as gradients of high to low land cover types (forested, agricultural, and developed). Water column nutrient data was collected from each reservoir and laboratory bioassays were conducted to determine: 1) the limiting nutrient status of each reservoir, and 2) if the TN:TP ratio and/or land cover could be used to predict nutrient limitation. From the bioassay study it was found that there was primary (greater significant chl-*a* in a treatment relative to the control) as well as secondary (greater significant chl-*a* in a treatment relative to all other treatments) limitation in Oklahoma reservoirs. Nitrogen and phosphorus primarily co-limited algal biomass in 15 reservoirs, while N and P were the sole limiting nutrient in only one reservoir each. Iron was important as a secondary co-limiting nutrient when it was added in combination with both N and P in three reservoirs. Positive correlations were found between the percentage of agriculture land cover in the watershed and water column nutrient concentrations (TN and TP), and N-limited reservoirs had significantly more agricultural in their watershed, but significantly less forest compared to P limited reservoirs. The TN:TP ratios were able to correctly predict 56% of the reservoirs limiting nutrient when compared to the results of the bioassays. Combined, these results highlight the importance of including multiple elements in eutrophication research. However, additional work on the interactive effects of iron with other nutrients is needed, as it may be important for effective management of eutrophication.

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CHAPTER I

INTRODUCTION

Eutrophication occurs when nutrients, studied mainly as nitrogen (N) and phosphorus (P), are available in overabundance and stimulate the growth of algae and other aquatic plants (Stoermer and Smol, 1999; Bennett *et al*., 2001). This term originally was used in relation to oligotrophy, which describes systems with little to no algal growth (Hutchinson, 1969). While eutrophication is a natural process of aging that occurs over time, the influences of human activities have increased this process and thus it can become problematic. Eutrophication has resulted in an increase in the frequency and intensity of nuisance algal blooms, reductions in dissolved oxygen concentrations, and the destruction of habitat for animals and other plants (Smith, 2003). These negative effects impact aquatic organisms, such as fish kills that result from anoxic conditions due to low dissolved oxygen and high concentrations of ammonia in the water, pollution of drinking water, decreased water clarity, and health risks to humans and/or animals due to increases in phytoplankton species, specifically N_2-fixing species of cyanobacterial blooms, that are toxic (Smith *et al*., 1999; Smith, 1998, Havens *et al*., 2002; Moss *et al*., 1997; Paerl *et al.*, 2001).

Historically, attempts to control eutrophication have focused on decreasing the amount of P that enters a waterbody (Alimov and Golubkov, 2014). Phosphorus and algal biomass (generally measured as chlorophyll *a*; chl-*a*) are often positively correlated in lakes and reservoirs (Canfield and Bachmann, 1981). For example, Jones and Bachmann (1976) found a strong positive correlation between the average chl-*a* concentration and total P (TP) in 143 lakes. Based on these relationships and a large body of research on the effects of P on algal biomass, freshwater systems have historically been considered to be P limited (Smith, 2003; Sterner, 2008). However, while N has long been thought to be a secondary nutrient to P in limiting algal growth, recent research suggests that it plays a larger role than once realized. Specifically, co-limitation of N and P may be of greater importance than originally thought (North *et al.*, 2007; Harpole *et al.*, 2011). Harpole *et al.* (2011) found in a meta-analysis of 641 studies across freshwater, marine and terrestrial systems where N and P were added, that algal biomass increased more when both nutrients were added compared to when N or P were added alone.

While eutrophication management has focused almost exclusively on N and P, there are other elements that have the potential to limit algal growth especially when N and P are available in high concentrations. Iron (Fe) is one of the most important essential elements for plant growth as it plays an important role in the process of photosynthesis and the synthesis of chlorophyll (Raven, 1988), and the amount of Fe can have an effect on how much P is necessary for algal growth, as P limited algae had twice the amount of Fe as those which are not limited (Raven, 1988; Chowdhury, 2014). In addition, iron also affects important chemical reactions in freshwater systems through the oxidation of ferrous iron (Fe^{2+}) into ferric iron (Fe^{3+}) in which H_2PO_4- anions act as a catalyst to the oxidation reaction which then reacts with Fe to create a non-soluble form of P and an oxidized form of Fe (Han *et a.l*, 2015; Stumm and Lee, 1961; Weiss, 1935). When iron is reduced it not only releases P from the sediment, but it also releases ferrous iron which can drive an increase in cyanobacteria (Molot *et al.*, 2014). As such, the importance and occurrence of Fe limitation should vary based on the availability of other nutrients including P. Vrede and Tranvik (2006) showed that while P appeared to be the limiting nutrient in northern oligotrophic lakes, co-limitation by Fe and P was also observed, showing that Fe has the potential to play an important role at least under some conditions (Chang *et al.*,, 1992; North *et al*., 2008; Xing and Liu, 2011). As such, there is growing interest to better understand how Fe affects algal growth in freshwater ecosystems (Molot *et al*., 2014; North *et al.*, 2007).

Understanding what nutrient or combination of nutrients limits algal growth is an important step in effectively managing eutrophication in a system. Although nutrient addition bioassays are often used (Dierberg, 1993; Dzialowski *et al.*, 2005), these can be time consuming and not feasible. Stoichiometry provides a framework for assessing nutrient limitation in aquatic systems as was seen by Redfield (1958) who found that the ratio of N:P in seston collected from the oceans was 16:1 stoichiometrically. The Redfield ratio has since been used to predict which nutrient is limiting, as a ratio lower than 16:1 suggests N limitation while a ratio greater than 16:1 suggest P limitation (Flett *et al*., 1980). Nutrient ratios are often used to predict nutrient limitation based on modifications of the 16:1 ratio presented by Redfield (Søndergaard *et al.*, 2017). For example, Dzialowski *et al*. (2005) used water column TN:TP ratios to correctly classify the nutrient limiting status of 88% of the reservoirs they studies, were P limited reservoirs had TN:TP ratios of > 30, reservoirs that were N-limited had TN:TP ratios of <9, and reservoirs that were co-limited by N and P had TN:TP ratios between 9 and 21. However, it is important to note that N:P ratios alone do not always accurately predict which nutrient is limiting in an aquatic system (Kobayashi and Church, 2003; Maberly *et al*., 2002; James *et al*., 2003). In a study done by Nikolai and Dzialowski (2014) of a large eutrophic reservoir, they found that the water column TN:TP ratios were only able to correctly predict the limiting nutrient from corresponding bioassay experiments 43% of the time.

Another potential tool for determining the nutrient limiting status of a system in the absence of bioassay studies is by examining what land use characteristics make up the surrounding watershed of a reservoir. Vanni *et al.* (2001) showed the export rates of nutrients (TN and TP) and nutrient ratios (N:P) were both higher in a lake where watershed land use was ~80% agricultural as compared to a lake which had a ~80% forested watershed. A large body of research has studied relationships between water quality and land use characteristics of watersheds and shown that it can be severely degraded within a relatively short period of time in watersheds that have strong human impacts as well as lead to negative water quality effects of lakes and reservoirs (Rast and Thornton, 1996; Alimov and Golubkov, 2014; Beaver *et al.*, 2014; Sharpley *et al.*, 1989). For example, watersheds that are dominated by cropland often export excess N and P from land applied fertilizer to lakes and reservoirs from flow through the soil (Arbuckle and Downing, 2001; Coulter *et al.*, 2004). Jones *et al*. (2004) showed there were positive correlations between TP and TN concentrations in Missouri reservoirs and the percent of cropland in their watersheds. Similarly, Missouri streams that had watersheds with a higher percentage of cropland contributed greater nutrient inputs whereas forest-covered watersheds contributed the smallest inputs of nutrients into the stream (Perkins *et al*., 1998). Furthermore, Carter and Dzialowski (2012) showed that not only the surface water column concentration of TP, but also the amount of TP that was released from anoxic sediment cores could be predicted from the percentage of cropland in the watershed of 25 mesotrophic to hypereutrophic reservoirs.

Relative to agricultural lands, forested watersheds generally release less N and P into adjacent waterbodies because plants are able to uptake nutrients from the soil and store it for use; this is one of the reasons why riparian zones are important in relation to agricultural watersheds (Mander *et al.*, 2005). Lenat and Crawford (1994) found that when compared to a site with a mostly agricultural watershed, the site with the mostly forested watershed had much lower nutrient concentrations. This was also seen by Crosbie and Chow-Fraser (2011) where they found that when comparing different watershed makeups, the watersheds which were mostly forested produced clearer, nutrient poor water compared to agricultural watersheds. While nutrient deficiency can possibly be a common problem that can be seen in forested areas, if N saturation occurs due to constant addition of excess nitrogen into the system, then the uptake capacity for the plants will be reached and that is where the excess leaches into the soil and thus N can become runoff for the watershed (Hunsaker *et al.*, 1995; Stoddard, 1994). However, watersheds that have a larger forested area are also likely to have a better buffer zone for the lake due to the runoff being stopped by herbaceous and/or rocky material then agricultural land, and a non-N saturated forested watershed would not exhibit this runoff.

Therefore, examining the amount of N in reservoirs mainly surrounded by forested areas can also be a good indication to the health of the forest as well.

Land cover within a watershed also has the potential to influence the movement of Fe into lakes and reservoirs as Fe ions were found to be in higher concentration in watershed areas where there was mostly undeveloped forest land (Carlson, 2014). An increase in acidity in soil has been seen to also lead to an increase in Fe, which could possibly explain why forested watersheds exhibit higher iron concentrations, along with considering the possible permeability of the soil (Das *et al.*, 2009; Jobbagy and Jackson, 2003; Carlson, 2014). However, the relationship between land cover and Fe in the water column is largely unknown.

Based on the strong impacts that watershed characteristics can have on nutrient concentrations in receiving waters, it is possible that these characteristics can be used to predict nutrient limitation. Jones *et al.* (2001) was able to use nutrient yield and landscape metric data to create landscape models in order to explain variations in nutrient loading from different qualities of watersheds in the Chesapeake Bay Basin, and this study found that agricultural land cover accounted for 50% of the variation of total nitrate and forested land cover accounted for 47% of the variation in total phosphorus. This relates to Vanni *et al* (2001) finding of higher agricultural watershed releasing more nutrients into the water body than a watershed with higher forested area. Since it is known that land cover affects nutrient concentration, it can be deduced that differences in land cover should also play a role in determining what nutrient is limiting a specific body of water.

The purpose of this study was to study nutrient limitation in a group of reservoirs representing a gradient in water quality. The first objective was determining if, and under what conditions, Fe limitation occurred in the reservoirs. This was done by conducting nutrient bioassay experiments with water collected from 25 Oklahoma reservoirs to determine how N, P, and Fe individually and in combination affected algal biomass. This represents a realistic representation of possible limitations in the water column. The second objective was wanting to determine if relatively easy to collect variables including nutrient ratios and watershed characteristics (e.g. percent agriculture and forest) could be used to infer nutrient limitation. Combined, this study will allow for a better understanding to begin with looking at what role different nutrients play in limiting algal biomass and what tools can be used to predict nutrient limitation.

CHAPTER II

METHODS

Reservoir Selection

A total of 25 reservoirs were selected in Oklahoma to represent a gradient in land cover and water quality. Land cover data for approximately 130 reservoirs were obtained from the National Land Cover Database (Fry *et al.*, 2011) and analyzed in ArcGIS (ESRA, 2011). The watershed for each reservoir was delineated using US Geological Survey Hydrological Unit Code (HUC) 12 data. Once the watersheds were defined, the percent land cover for each major land-use type (e.g. cropland, water, forest, etc.) was calculated using the tabulate area function in ArcGIS 10 Spatial Analyst tools. Two land use categories were used to select reservoirs which included the combination of crop and hay pasture, which will be referred to as agricultural, which are generally associated with poor watershed quality, and combined forested area made up of deciduous, evergreen, and mixed forest, which will be referred to as forested, which are generally associated with higher watershed quality (Arbuckle and Downing, 2001; Coulter *et al.*, 2004; Crosbie and Chow-Fraser, 2011). The reservoirs were then grouped based on forested watershed percentage to be "High" (81-49%), "Medium" (49-21%) and "Low" (20-2.0%).

Percent forest was used to group the reservoirs into these categories because of the negative relationship between nutrient influx and forest cover (Lenat and Crawford, 1994). The percentages used to group these watersheds were loosely based on Vanni *et al.* (2011), as their study involved three lakes which had watersheds that were 83.2, 46.2, and 12.4% forested to show low to high nutrient input, respectively.

Field Sampling and Elemental Analyses

At each reservoir 50 L of surface water was collected and brought back to the lab for analyses of total nutrient concentrations and for use in the bioassay experiments within 48 hours of collection. One-L was placed in a brown bottle and stored at 4°C, and 1-L was placed in a brown bottle and acidified with sulfuric acid (2%) and stored at 4°C as well. Colorimetric methods were used to determine the total concentrations of TN and TFe in the water column using a HACH© DR5000 UV-VIS spectrophotometer using Hach© methods 10071 and 8008, respectively. Total P was determined spectrophotometrically after persulfate digestion (Ebina *et al.*, 1983). The amount of TP was also used to determine the trophic state of each reservoir using the criteria presented in Nürnberg (1996), which states water bodies with TP < 30 ug/L are mesotrophic, TP = 30-100 ug/L are eutrophic and TP > 100 ug/L are hypereutrophic.

Bioassays

Bioassay experiments were started within 48 hours of collecting the reservoir water. The reservoir water was stored in an environmental chamber set at 20°C until used for the bioassays. Approximately 40 L of reservoir water was poured through a 243 µm mesh filter in order to remove any plant material and macrozooplankton from each reservoir sample. A factorial design was created where each of the three nutrients are added to 1 L bioassay bottles individually and in all possible combinations (8 treatments per reservoir – control with no added nutrients, +N, +P, +Fe, +NP, +NFe, +PFe, +NPFe). Each treatment was replicated in triplicate bioassay jars for a total of 24 bottles per reservoir. Nutrients were added as 1600 µg L^{-1} of N as KNO_3, 100 µg L^{-1} of P as K_2HPO_4, per Redfield (1958) and 1 mg/L of Fe, representing the amount in algal culture media (Kilham *et al.*, 1998). The bioassay jars were placed in an environmental chamber that was set at 20°C, on a 16:8 hour light:dark cycle. This temperature and light cycle were chosen to mimic conditions commonly used in previous algal-nutrient bioassay studies (Dierberg, 1993; Dzialowski *et al.*, 2005). Algal biomass was measured immediately from the water, as well as daily from each jar for five days using a Turner Designs Trilogy Fluorometer (Model 7200-000; Sunnyvale, CA) based on preliminary bioassays showing the algal responses to nutrients occur during this period (unpublished data). Relative fluorescence (RFU) is often used as a surrogate for algal biomass in bioassay experiments (Peterson *et al.*, 1983) and our own laboratory studies show that there are strong positive correlations between fluorescence and chl-*a* (unpublished data). Bioassay experiments were conducted for approximately 3 reservoirs at a time and all bioassays were completed between the months of June and August of 2016.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was used to test for differences in the algal growth (as measured as relative fluorescence) in the different nutrient treatments from the bioassay results. Tukey's post-hoc tests ($P<0.05$) were used to determine which treatments differed from the controls when significant treatment effects are determined through the ANOVA. In cases where the data did not meet the assumption of the ANOVA (normal distribution and/or homogeneity of variance) data were either log transformed or a Kruskall-Wallis non-parametric ANOVA on ranks with Tukey's post-hoc tests were used. The results of the ANOVA and post-hoc comparisons were used to determine which nutrient was limiting in each reservoir. Based on Nikolai and Dzialowski (2014), single nutrient limitation was inferred when fluorescence in the single nutrient treatment (e.g., N, P, or Fe) was greater than the control. Similarly, when two-nutrient combinations (e.g., +NP, +NFe, +PFe) were greater than the respective single nutrient treatments, then the two nutrients were considered co-limiting. Finally, if the fluorescence after addition of all three nutrients (+NPFe) was greater than individual or dual supplementation (i.e. +NP, +NFe, +Fe, +P, +N), then all three elements were inferred to be co-limiting. This above procedure was also done to determine primary and secondary limitation, where the first significant fluorescence value relative to the control was deemed the primary limiting nutrient(s), and then if there is a significant increase in fluorescence relative to the primary limiting nutrient(s), that treatment is deemed the secondary limiting nutrient.

Linear regressions were used to assess relationships between water quality and land use. R^2 values and equations were recorded from a best fit analysis in SigmaStat to see the strength in relationships between all variables. TN:TP; TN:TFe, and TP:TFe ratios were recorded from total concentrations of the nutrients from the water column. All variables including percent watershed cover, total nutrients, and ratios were compared by a best fit analysis to consider relationships. The 25 reservoirs were grouped based on percent forest in their watershed (High, Medium, Low) and all of the total nutrient concentrations and ratios were compared using a one-way ANOVA with a Tukey post-hoc test ($P<0.05$). The primary limiting nutrient and the percentage of each watershed type were then compared using a One-way ANOVE with a Tukey-post hoc test ($P<0.05$) to examine relationships between primary nutrient limitation and percent watershed makeup.

CHAPTER III

RESULTS

General Water Quality and Trophic State

The Oklahoma reservoirs showed a wide range in chl-*a* and total nutrient concentrations. Chl-*a* concentrations averaged 188 RFU and ranged between 83-364 RFU (Table 2). Significant positive relationships existed between chl-*a* and both TP and TN in the reservoirs (Figure 4). However, the relationship between chl-*a* and TN had a higher R^2 value than the relationship between chl-*a* and TP (Figure 4). No significant relationship existed between TFe and chl-*a* (Figure 4). Total Fe concentrations averaged 975 µg/L and ranged between 7424-161 µg/L (Table 2). Total nitrogen concentrations averaged 680 µg/L and ranged between 100-1900 µg/L (Table 2). Total Phosphorus concentrations averaged 84 µg/L and ranged 359-24 µg/L (Table 2). Based on the TP values used to determine trophic state in Nürnberg (1996), two of the reservoirs were classified as mesotrophic (TP >30 µg/L), 17 were classified as eutrophic (TP= 30-100 µg/L), and six were classified as hypereutrophic (TP<30 µg/L) (Table 4).

Bioassay

With respect to the treatment that showed the greatest increase in chl-*a* relative to the control and the other treatments, 20 reservoirs were determined to be NP co-limited, three NPFe co-limited, one N limited and one P limited (Figure 2). When further looking at nutrient limitation to see what other treatments increased algal biomass (e.g., primary and secondary limiting nutrient; see Methods), it was determined that there were four N, six P and 15 NP primarily co-limited reservoirs (Figure 3). Three of the N primarily limited reservoirs were further classified as secondarily NP co-limited (e.g., the NP treatment had significant higher chl-*a* values compared to both control and N treatments), and five of the primarily P limited reservoirs were further classified as secondarily NP co-limited (e.g., the NP treatment had significant higher chl-*a* values compared to the control and P treatments) (Table 3). This resulted in eight reservoirs that were secondarily NP co-limited (Table 3). Three reservoirs were NPFe secondarily co-limited. Another three reservoirs were NP primarily limited, and secondarily limited by NPFe, where algal biomass showed an increase in chl-*a* in the NPFe treatment relative to the control as well as the NP treatment (Table 3). The only N limited reservoir, El Reno, had the highest TP value (359 µg/L) while the only P limited reservoir, Pine Creek, had the second highest TP value (211 µg/L), yet also had the highest TFe value (7424 µg/L) (Table 2). Neither became NP secondarily co-limited with the addition of P and N, respectively (Table 3).

The limiting nutrient that was expected based on the comparisons between the measured TN:TP ratios from the reservoirs and the ranges of TN:TP ratios presented by Guildford and Hecky (2000) to predict limiting nutrients identified the primary limiting nutrient in only 14 of the 25 reservoirs (56%) (Table 3). Most were predicted to be N limited by the ratio (<9), however the bioassay study indicated that they were primarily NP co-limited, meaning there was no significant increase in chl-*a* in the N or P treatments relative to the control, yet there was significance between the NP treatment relative to the control.

Relationships between land cover, nutrient limitation, and water quality

Nutrient concentrations and ratios did not differ between the three groups of reservoirs based on the amount of forest in their watershed (ANOVAs for all variables comparing low, medium, and high forest reservoirs $P>0.05$; Table 5). However, there were several significant linear relationships between the percentage of land cover types and nutrient concentrations. There was a significant negative relationship between $\log_{10}$TN and the percentage of forest in the watershed (Figure 5). In contrast, $\log_{10}$TP and $\log_{10}$TFe did not increase with increasing forest in the watershed (Figure 5). Log_{10}TN and $\log_{10}$TP increased significantly with increasing agriculture in the watershed (Figures 6). There was not a significant relationship between $\log_{10}$TFe and agriculture in the watershed (Figure 6). Similar trends were observed between $\log_{10}$developed in the watershed and total nutrient concentrations. Log_{10}TN showed a significant increase with increasing percent $\log_{10}$developed in the watershed (Figure 7). However, $\log_{10}$TP and $\log_{10}$TFe showed no significant relationship with the percent $\log_{10}$developed in the watershed (Figure 7).

From the ANOVA ran between the primary limiting nutrient of the system and the land cover, it was found that there was a significant increase in percent agricultural land cover in watersheds which had N limited reservoirs, and a significant increase in the percent forested land cover in watersheds with P limited reservoirs (Figure 8). There were no significant differences between limitations with developed land cover (Figure 8). Reservoirs that were N limited had significantly more agriculture, but less forest in their watershed compared to P limited reservoirs (ANOVA, Tukey's $P<0.05$). There were no differences in the land cover between reservoirs that were NP limited and those that were either P or N limited for both agricultural and forested.

Table 1. Total percentages of different land cover types in the watershed of each reservoir which were taken from the Oklahoma Water Resource Board (OWRB) data. Developed includes the sum of open space and low, medium and high intensity developed. Forested includes the sum of deciduous, evergreen, and mixed forest. Agricultural includes the sum of hay pasture and cultivated crops. Wetland includes the sum of woody and emergent herbaceous.

Reservoirs	Open Water	Developed	Barren Land	Forested	Shrub Scrub	Herbaceous	Agricultural	Wetland
American Horse	1.7	3.6	0.0	17.0	0.0	50.3	27.3	0.0
Ardmore	2.3	11.2	0.0	23.3	0.0	37.9	25.3	0.0
Atoka	9.4	4.8	0.4	54.9	2.9	13.2	13.3	1.1
Broken Bow	14.1	3.2	0.0	78.2	1.6	2.0	0.4	0.5
Canton	16.7	4.3	0.2	12.6	0.0	31.3	34.3	0.7
Carl Albert	1.4	5.4	0.0	73.9	2.2	6.7	9.9	0.5
Clinton	0.9	6.3	0.0	20.8	9.9	31.3	30.3	0.6
Crowder	1.0	4.6	0.0	2.0	0.0	47.1	45.2	0.0
Durant	1.8	5.4	0.2	21.8	0.0	48.0	22.7	0.2
El Reno	2.5	16.3	0.0	3.5	0.0	24.1	53.5	0.0
Eufaula	15.5	6.0	0.2	37.6	0.9	18.8	19.8	1.2
Hudson	6.2	7.1	0.1	35.0	0.6	21.7	28.9	0.4
Hulah	5.7	4.2	0.0	29.0	0.0	40.6	20.5	0.1
Jean Neustadt	2.9	5.4	0.1	18.9	0.0	44.2	28.4	0.0
Lugert-Altus	6.2	4.1	2.3	9.5	32.7	5.8	35.4	4.1
McGee	4.3	1.7	0.0	61.8	5.9	17.7	8.3	0.3
Pine Creek	3.8	5.2	0.0	41.1	8.8	21.7	18.4	1.0
Sahoma	2.4	7.8	0.0	35.2	1.1	30.8	22.5	0.2
Sardis	11.1	2.0	0.0	62.2	2.8	6.1	14.3	1.4
Shell	5.8	7.6	0.2	63.0	0.0	17.6	5.7	0.0
Talawanda No. 1	4.9	4.0	0.5	54.5	1.2	19.3	15.2	0.3
Talawanda No. 2	4.9	4.0	0.5	54.5	1.2	19.3	15.2	0.3
Vanderwork	0.9	5.0	0.0	3.5	28.0	23.9	45.6	0.0
Wayne Wallace	0.9	3.8	0.0	80.5	0.9	3.9	9.0	0.9
Wetumka	3.2	5.2	0.3	49.1	0.0	24.1	18.1	0.0

Table 2. Total nutrient concentrations, starting chl-*a* (measured as relative flourescence, RFU), and nutrient ratios for the 25 Oklahoma reservoirs.

Reservoir	TP (ug/L)	TFe (ug/L)	TN (ug/L)	Starting *chl-a* (RFU)	TN:TFe	TP:TFe	TN:TP
American Horse	38	198	400	183	2.02	0.19	10.67
Ardmore	25	161	400	104	2.48	0.16	16.00
Atoka	102	1327	700	149	0.53	0.08	6.84
Broken Bow	24	285	100	83	0.35	0.08	4.18
Canton	67	632	800	168	1.27	0.11	11.99
Carl Albert	35	260	400	92	1.54	0.13	11.49
Clinton	72	744	800	218	1.08	0.10	11.13
Crowder	64	372	600	348	1.61	0.17	9.36
Durant	31	360	600	195	1.67	0.09	19.54
El Reno	359	186	1900	244	10.22	1.93	5.30
Eufala	39	347	300	191	0.86	0.11	7.67
Hudson	174	409	1300	230	3.18	0.43	7.47
Hulah	207	1612	800	364	0.50	0.13	3.87
Jean Neaustadt	59	2269	700	133	0.31	0.03	11.78
Lugert-Altus	79	384	700	201	1.82	0.21	8.84
McGee	35	422	300	108	0.71	0.08	8.57
Pine Creek	212	7424	1000	165	0.13	0.03	4.72
Sahoma	55	409	800	303	1.96	0.13	14.60
Sardis	46	484	300	96	0.62	0.10	6.49
Shell	40	198	600	234	3.02	0.20	14.93
Talawanda No.1	39	620	600	163	0.97	0.06	15.46
Talawanda No.2	34	1054	200	120	0.19	0.03	5.87
Vanderwork	58	310	1000	258	3.23	0.19	17.21
Wayne Wallace	158	3360	1000	139	0.30	0.05	6.33
Wetumka	54	533	700	224	1.31	0.10	12.87

Table 3. Primary and secondary limiting nutrients (N=Nitrogen limited, P=Phosphorus limited, NP= Nitrogen and Phosphorus co-limited, NPFe= Nitrogen, Phosphorus, and Iron co-limited) from the bioassay study as well as what primary limiting nutrient was predicted from the TN:TP ratio based on criteria presented in Guildford and Hecky (2000) for 25 Oklahoma reservoirs. The primary limiting nutrient was found to be the treatment with the first significant increase in chl-*a* relative to the control, and the secondary limiting nutrient was found to be the treatment with an increase in chl-*a* relative to the primary limiting nutrient. The highlighted sections represent reservoirs in which the ratio was able to correctly predict the primary limiting nutrient.

Reservoir	Primary	Secondary	Predicted primary limiting nutrient(s) from TN:TP ratio
American Horse	NP		NP
Ardmore	NP		NP
Atoka	P	NP	N
Broken Bow	P	NP	NP
Canton	NP	NPFe	NP
Carl Albert	P	NP	NP
Clinton	NP		NP
Crowder	NP		NP
Durant	N	NP	NP
El Reno	N		N
Eufala	NP	NPFe	N
Hudson	P	NP	N
Hulah	N	NP	N
Jean Neaustadt	NP		NP
Lugert-Altus	N	NP	N
McGee	P	NP	N
Pine Creek	P		N
Sahoma	NP		NP
Sardis	NP		N
Shell	NP		NP
Talawanda No.1	NP	NPFe	NP
Talawanda No.2	NP		N
Vanderwork	NP		NP
Wayne Wallace	NP		N
Wetumka	NP		NP

Table 4. Reservoirs grouped as either high (80-49%), medium (49-21%), or low (20-2.0%) forested area based on the percent forest in their watershed. The trophic states of each reservoir are included based on their TP values and criteria presented in Nurnberg (1996).

Reservoir	Forested Group	Trophic State
American Horse	Low	Eutrophic
Ardmore	Medium	Mesotrophic
Atoka	High	Hypereutrophic
Broken Bow	High	Mesotrophic
Canton	Low	Eutrophic
Carl Albert	High	Eutrophic
Clinton	Low	Eutrophic
Crowder	Low	Eutrophic
Durant	Medium	Eutrophic
El Reno	Low	Hypereutrophic
Eufala	High	Eutrophic
Hudson	Medium	Hypereutrophic
Hulah	Medium	Hypereutrophic
Jean Neaustadt	Low	Eutrophic
Lugert-Altus	Low	Eutrophic
McGee	High	Eutrophic
Pine Creek	Medium	Hypereutrophic
Sahoma	Medium	Eutrophic
Sardis	Medium	Eutrophic
Shell	High	Eutrophic
Talawanda No.1	High	Eutrophic
Talawanda No.2	High	Eutrophic
Vanderwork	Low	Eutrophic
Wayne Wallace	High	Hypereutrophic
Wetumka	High	Eutrophic

Table 5. One-way ANOVA's results comparing the three forested groups (High=80-49%, medium=49-21%, low=20-2.0%) for all water quality variables.

Response Variable	**ANOVA – F value**	**P-value**
Total Nitrogen	F(2,22)= 1.935	0.168
chl-*a* (RFU)	F(2,22)= 2.572	0.099
Total Nitrogen:Total Phospohrus	F(2,22)= 0.141	0.869
Total Phosphorus	F(2,22)= 1.071	0.360
Total Iron	F(2,22)= 0.707	0.504
Total Nitrogen:Total Iron	F(2,22)= 1.755	0.196
Total Phosphorus:Total Iron	F(2,22)= 1.289	0.295

Table 6. Liner regressions between water quality variables and land cover data. R^2 and p-values are shown for the full data set. Highlighted data is significant ($p<0.05$) and chl-*a* values are in RFU (relative fluorescent units). (TP= Total Phosphorus, TN= Total Nitrogen, TFe= Total Iron).

Watershed	Variable	R^2	P-value	Coefficient	Slope
Forested	log_{10}TP	0.069	0.239	1.92	-0.320
	log_{10}TN	0.222	0.018	2.96	-0.536
	log_{10}TFe	0.041	0.333	2.61	0.347
	chl-*a*	0.290	0.005	252	-171
	log_{10}TN:TFe	0.235	0.014	0.349	-0.883
	log_{10}TP:TFe	0.175	0.037	-0.694	-0.667
	TN:TP	0.064	0.222	11.9	-4.64
Agricultural	log_{10}TP	0.161	0.047	1.58	0.965
	log_{10}TN	0.351	0.002	2.48	1.24
	log_{10}TFe	0.044	0.317	2.89	-0.657
	chl-*a*	0.285	0.006	118	311
	log_{10}TN:TFe	0.322	0.003	-0.409	1.89
	log_{10}TP:TFe	0.308	0.004	-1.31	1.62
	TN:TP	0.024	0.463	8.96	5.16
log_{10}Developed	log_{10}TP	0.096	0.132	2.43	0.482
	log_{10}TN	0.269	0.008	3.68	0.701
	log_{10}TFe	0.062	0.231	2.08	-0.507
	chl-*a*	0.116	0.096	356	128
	log_{10}TN:TFe	0.312	0.004	1.60	1.21
	log_{10}TP:TFe	0.273	0.007	0.348	0.989
	TN:TP	0.062	0.228	17.2	5.44

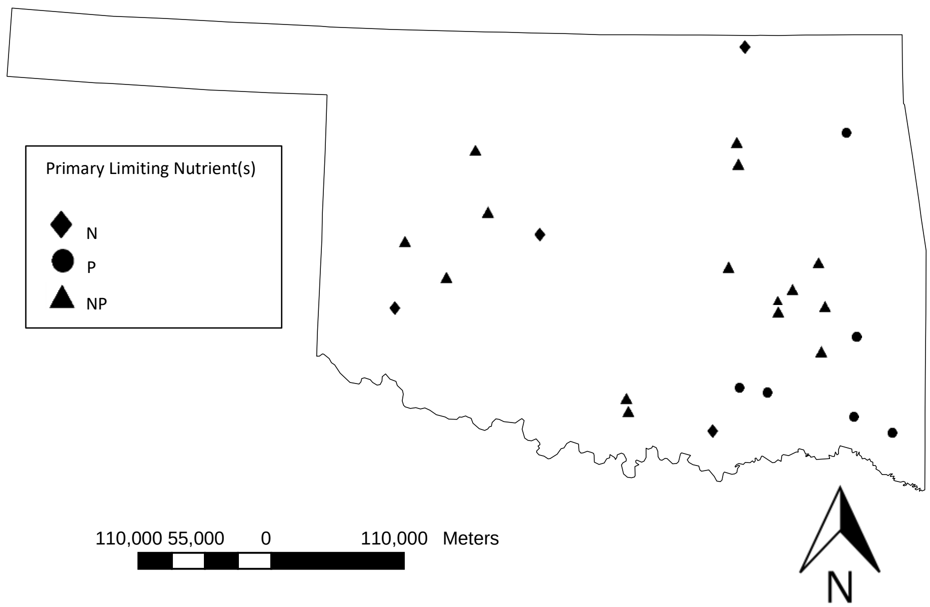

Figure 1. Location of study reservoirs and the primary limiting nutrient(s) of each reservoir as determined by the nutrient bioassay studies (N=nitrogen, P=Phosphorus, NP= Nitrogen and phosphorus). The reported limitation for each reservoir is the nutrient treatment that showed the first significant increase in chl-*a* (RFU) relative to the control.

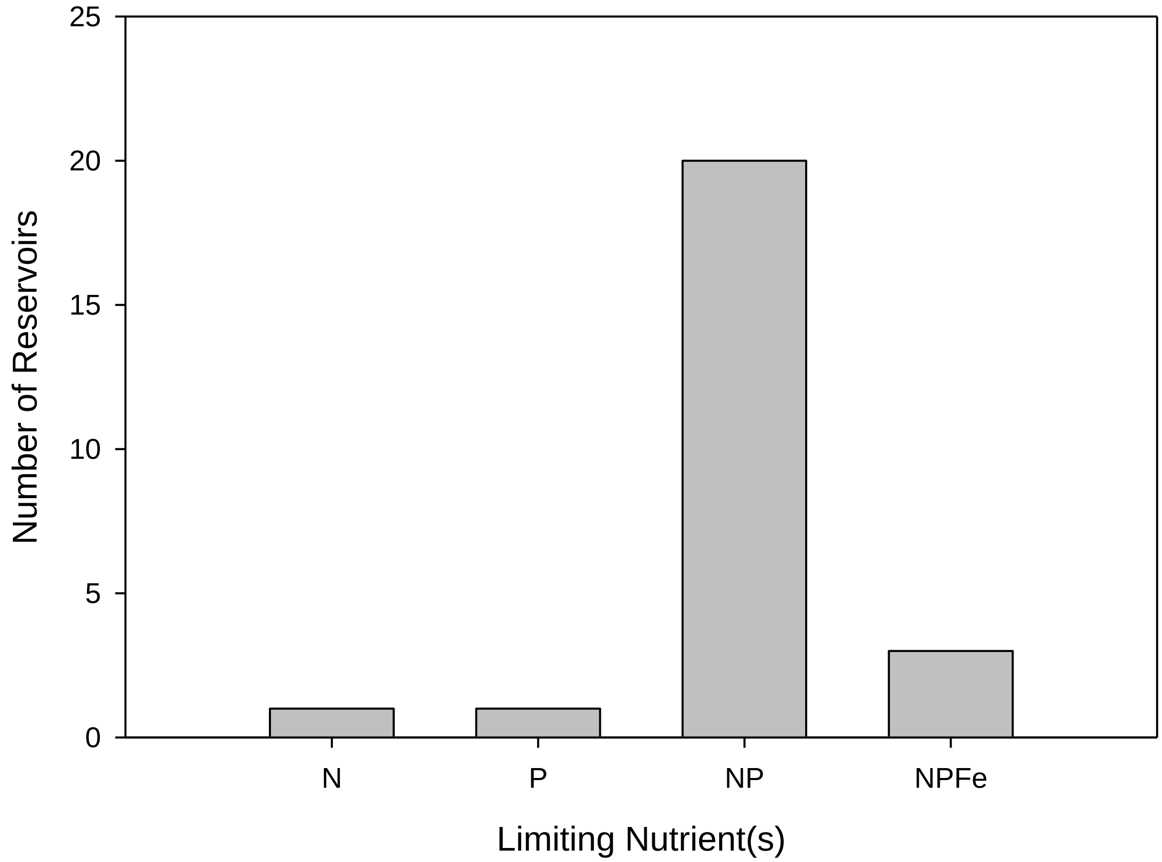

Figure 2. Number of reservoirs limited by the different nutrient treatments (N=nitrogen, P=phosphorus, NP=nitrogen and phosphorus, NPFe=nitrogen, phosphorus, and iron). This limiting nutrient was determined as the greatest increase in chl-*a* (RFU) values relative to the control and all other treatments. Note that only the treatments that were determined to be limiting nutrients in at least one bioassay are included in the figure.

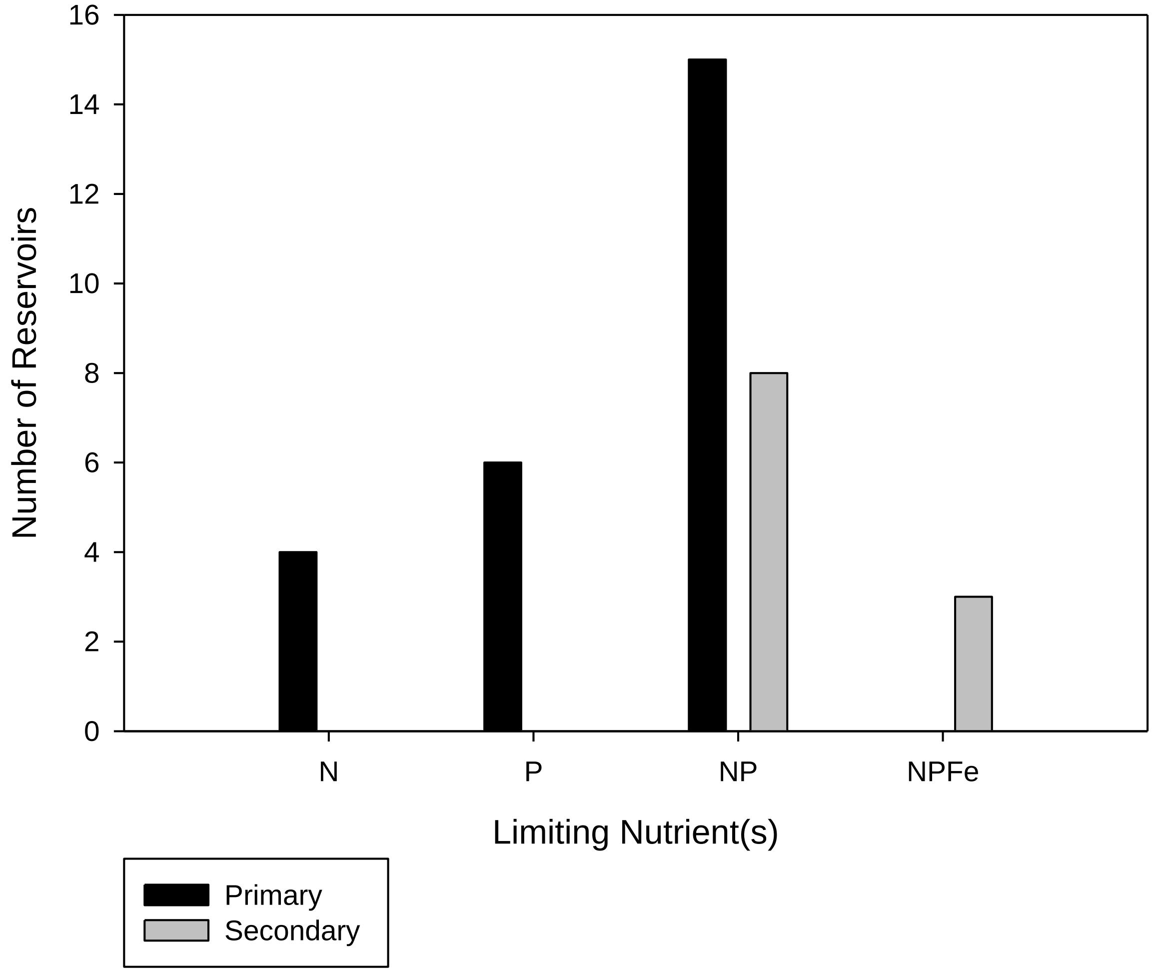

Figure 3. The primary and secondary limiting nutrients from the bioassay experiments (N=nitrogen, P=phosphorus, NP= nitrogen and phosphorus, NPFe= nitrogen, phosphorus, and iron). Primary limitation was determined for treatments that had significantly higher RFU values than the control, but not necessarily the other treatments. Secondary limitation was then determined as the treatment that had significantly greater RFU values relative to the primary limiting nutrient.

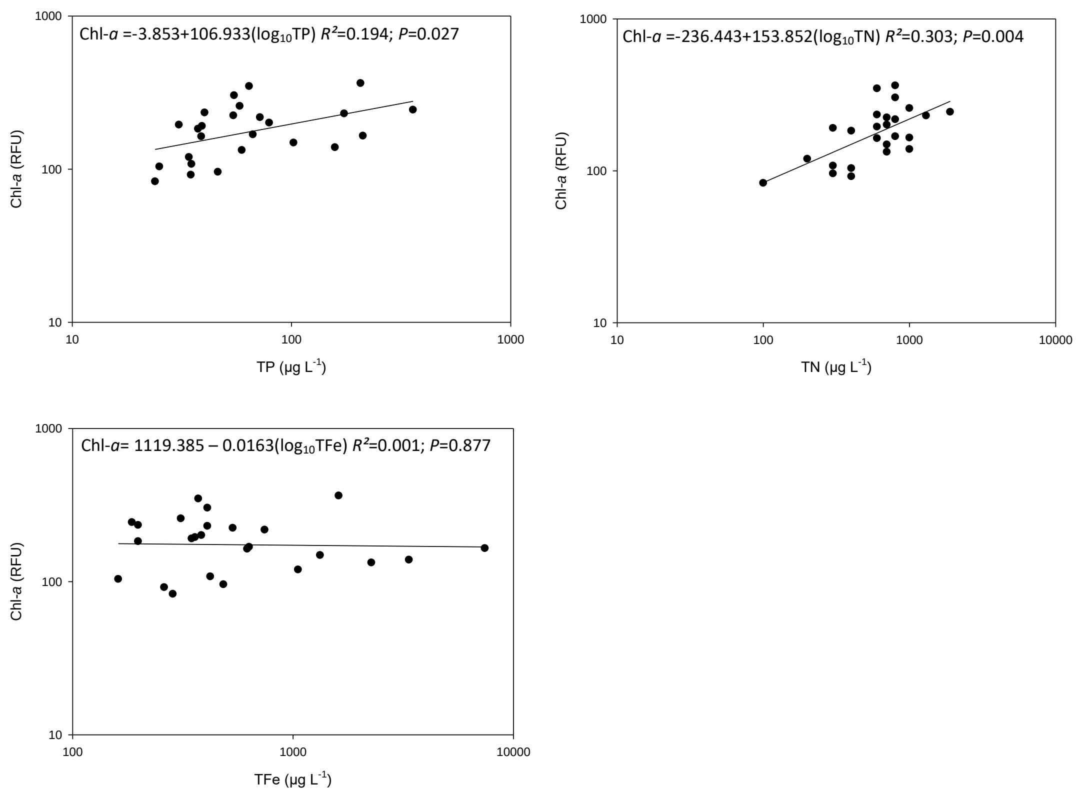

Figure 4. Relationships between chl-*a* in RFU (relative fluorescent units) and total nutrients (TN= total nitrogen; TP=total phosphorus; TFe=total iron) in the 25 sampled Oklahoma reservoirs.

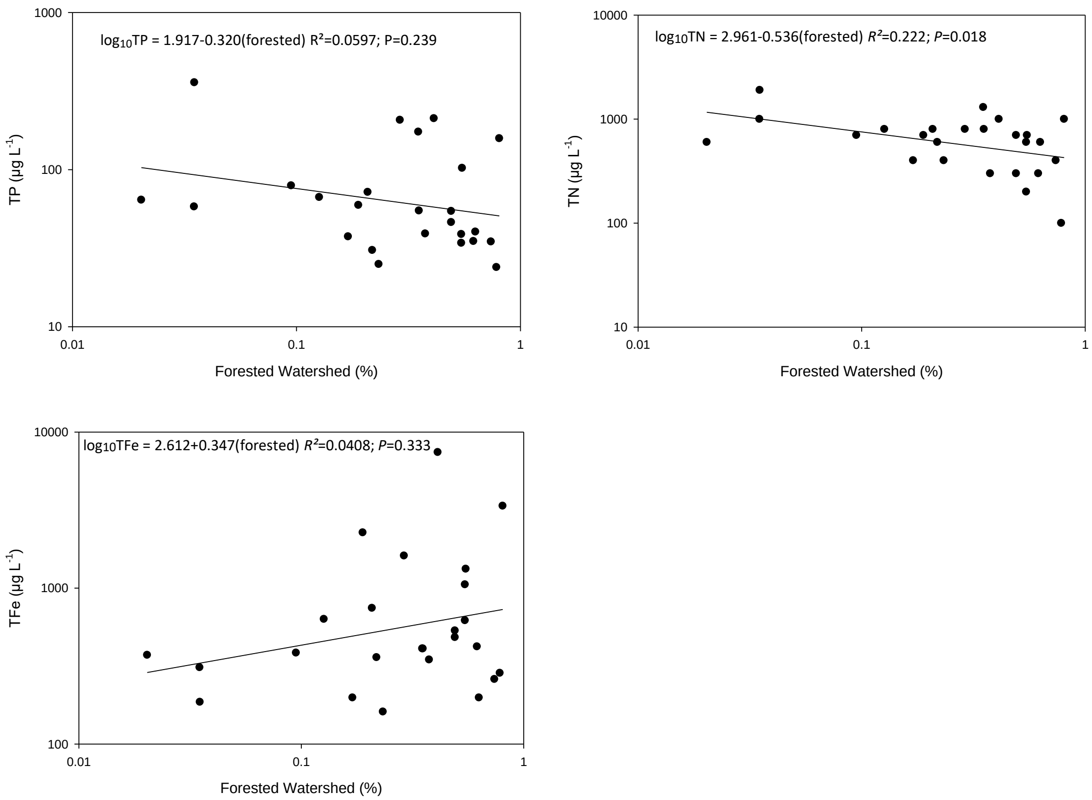

Figure 5. Relationship between percent forested land cover in the reservoirs watershed and total nutrient concentrations (TN=total nitrogen; TP=total phosphorus; TFe= total iron) in the 25 sampled Oklahoma reservoirs.

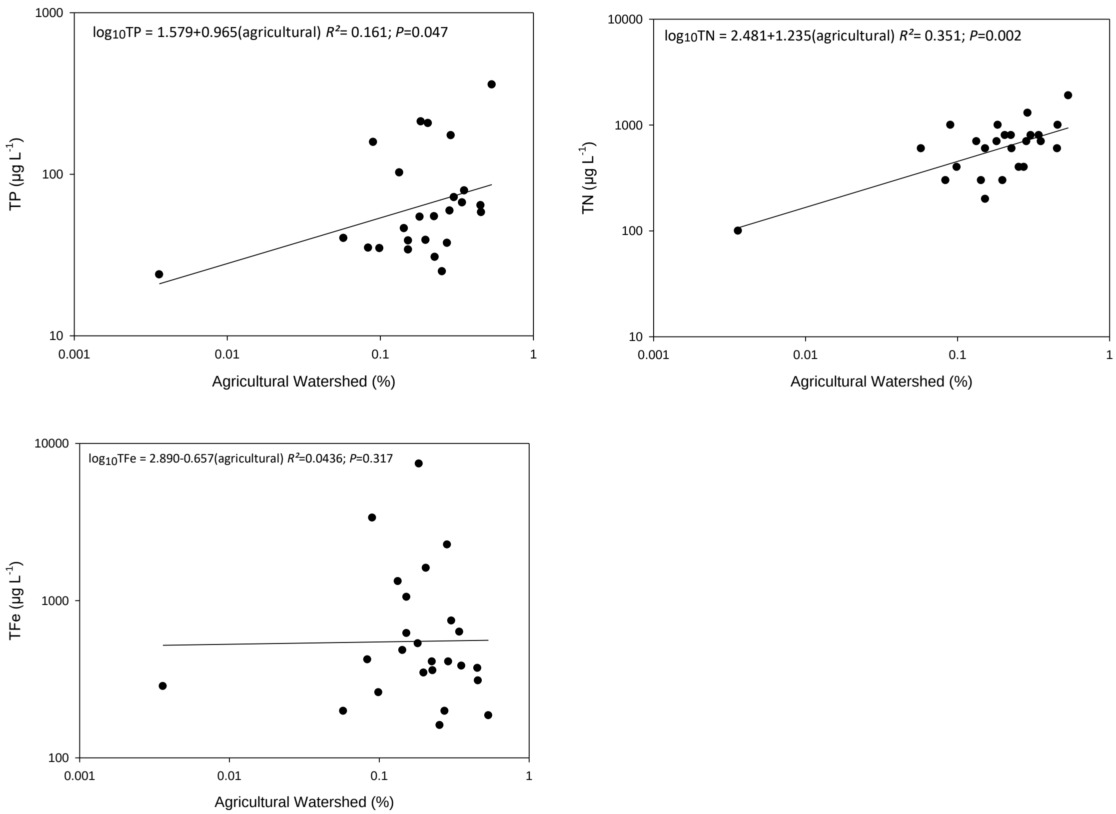

Figure 6. Relationship between percent agricultural land cover in the reservoirs watershed and total nutrient concentrations (TN=total nitrogen; TP=total phosphorus; TFe= total iron) in the 25 sampled Oklahoma reservoirs.

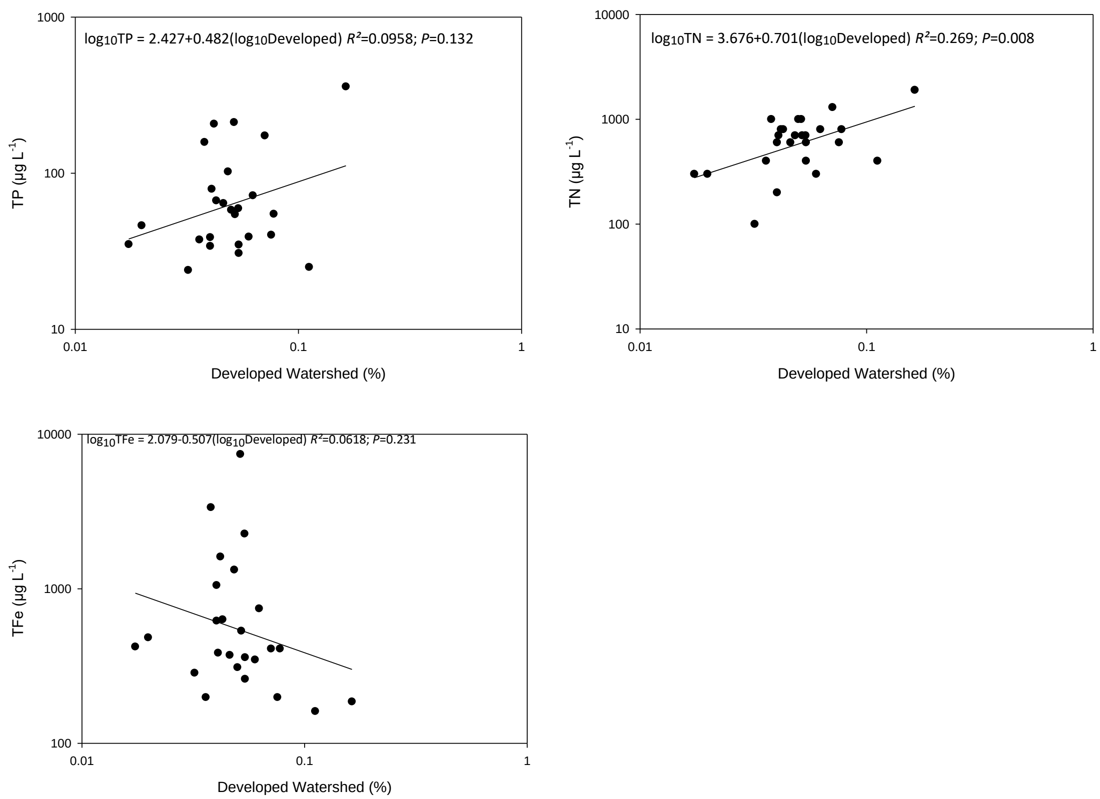

Figure 7. Relationship between percent developed land cover in the reservoirs watershed and total nutrient concentrations (TN=total nitrogen; TP=total phosphorus; TFe= total iron) in the 25 sampled Oklahoma reservoirs.

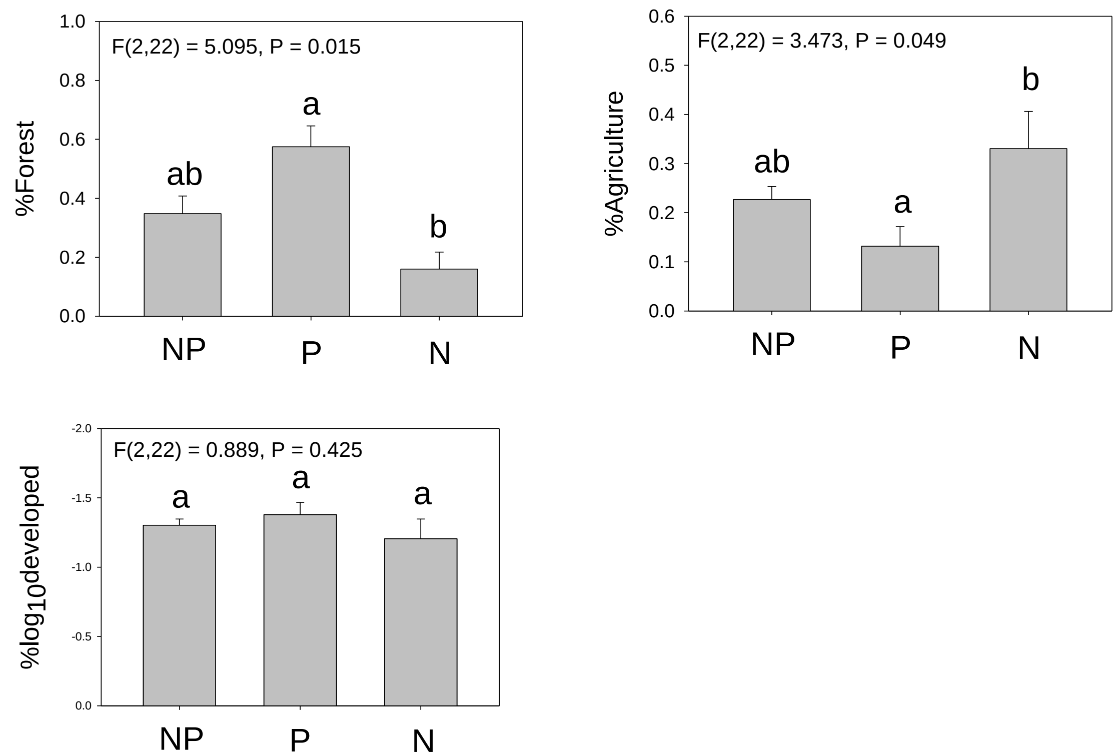

Figure 8. Differences in land cover between the primary limiting nutrient groups based on the results from the bioassays (Nitrogen (N), phosphorus (P), nitrogen and phosphorus (NP) limiting). P and N reservoirs differed in forested and agricultural land cover based on ANOVA and Tukey’s post-hoc tests. Different letters represent differences in treatments.

CHAPTER IV

DISCUSSION

Understanding which nutrients limit primary production, and the development of tools for predicting nutrient limitation, are important goals of lake and reservoir managers. In this study, bioassay experiments were conducted with water collected from 25 reservoirs, of which two were mesotrophic, 17 were eutrophic, and six were hypereutrophic. While there were significant relationships between both TN and TP and chl-*a*, the relationship was stronger for TN. Similarly, Abell *et. al* (2011) conducted a meta-analysis of 101 New Zealand lakes and found that TN had a stronger relationship with chl-*a* (r=0.85) than TP (r=0.80) and Søndergaard et. al. (2017) found that there were stronger relationships between TN and chl-a than there were between TP and chl-*a* in Danish lakes that had high P concentrations.

The results from the bioassays further highlight the importance of considering nutrients besides P, especially in eutrophic systems. For example, 16% of the reservoirs were primarily N limited and 60% of the reservoirs were primarily NP co-limited, while

24% were P-limited. These results are consistent with several studies showing the N limitation and N and P co-limitation occur more often than previously thought (Bergström *et al.,* 2008; Maberly *et al.*, 2002; Morris and Lewis, 1988; Nikolai and Dzialowski, 2014). For example, Maberly *et al.* (2002) found that for 30 lakes in Scotland and Norther Ireland 13% of lakes were N limited while 63% were co-limited by N and P. Morris and Lewis (1988) showed that in 8 Colorado lakes sampled multiple times throughout the season N limitation occurred 33% of the time and combined N and P limitation occurred 46% of the time. While controlling for P would help prevent the growth of algae for the P and NP limited reservoirs, it would not prevent algae growth for the N limited systems. With respect to Oklahoma, Nikolai and Dzialowski (2014) found that when doing nutrient bioassays over the course of four months (June-October) at four locations in a eutrophic reservoir, N and P co-limitation occurred in August while N limitation occurred in September. Our results combined with these previous studies highlight the potential importance of considering N and P and the potential role that they both play in eutrophication, especially in eutrophic or hypereutrophic systems that dominated the current study, and is generally representative of reservoirs in the Great Plains.

Interestingly, only two reservoirs were limited by a single nutrient (one P and one N). The N limited reservoir (El Reno) had the highest concentration of TP and relatively low TN:TP ratio. The watershed of this reservoir contains a golf course which potentially contributes nutrient rich runoff, and could help explain the large concentration of TP. The second highest concentration of TP was found in Pine Creek reservoir, and this was the only P limited reservoir as indicated by the bioassays. This reservoir also exhibited a very high concentration of TFe, which could possibly be an explanation for why it was P limited. It is likely that interactions with Fe in oxygenated waters decreases bioavailability of P. This process has been known to influence phosphorus (Mortimer, 1942) and precipitation of iron into the water column have been found to cause an increase of P sedimentation (Hongve, 1997), which under anoxic conditions would cause P to not be released into the water column.

The nutrient limitation bioassays also allowed a better understand how Fe influenced primary production in reservoirs. While Fe is important for photosynthetic processes and binding phosphorus in sediment, little is known about if it limits primary production (Raven, 1988; Chowdhury, 2014; Han *et al.*, 2015; Stumm and Lee, 1961; Weiss, 1935). In this study, it was found that Fe only increased algal growth when it was added in combination with both N and P and never alone. These results are supported by bioassay experiments conducted in a variety of habitats showing that Fe can co-limit algal production with N and/or P (Chang *et al.*, 1992; Sterner *et al.*, 2004; Moore *et al.*, 2006; North *et al.*, 2007). For example, Sterner *et al.* (2004) and North *et al.* (2007) found that Fe additions alone never promoted algal growth in Lake Superior and Erie, respectively, but they did increase algal biomass when Fe was added in combination with N and P. These studies suggest that Fe additions have the greatest potential to affect algal biomass in systems that have high inputs of N and/or P. Sampling from a wider variety of freshwater systems with differing nutrient concentrations may be beneficial to further understand how Fe interacts with N and P and when co-limitation is most likely to occur.

It is also important to note that this study only looked at how Fe affected bulk measurements of algal biomass. However, Fe may increase the biomass of individual species such as cyanobacteria. For example, Fe makes up a large proportion of the enzyme nitrogenase, which is used by cyanobacteria to convert atmospheric nitrogen into a bioavailable form through nitrogen fixation (Hoffman *et al.*, 2014) and Molot *et al.* (2014) developed a model predicting that Fe^{2+} (ferrous iron) release from sediments under anoxic conditions was a main driver of cyanobacteria production in freshwater systems. In support, Hyenstrand *et al.* (2000) found that adding Fe into enclosures with N and P caused a greater increase in cyanobacteria than the enclosures without Fe. Preliminary research from a eutrophic reservoir in Oklahoma also showed that while Fe additions did not affect total algal biomass, they did increase the abundance of cyanobacteria to a point (P. Lind, unpublished data). Additional research is therefore needed to better understand how Fe influences cyanobacteria production and under what conditions it may cause blooms.

In the absence of bioassay studies, it has been proposed that TN:TP ratios can be used to predict nutrient limitation. While this was originally based on the Redfield ratio, others have developed ranges of ratios based on the fact algal species differ in the ratios that they require for growth and previous attempts to use TN:TP ratios have produced mixed results (Smith, 1982; Søndergaard *et al.,* 2017; Rhee, 1978). Using the ratios presented by Guildford and Hecky (2000), the TN:TP ratio was able to correctly predict the limiting nutrient in 56% of reservoirs studied here. Our study further suggests that these ratios should be used with caution. Studies which aim to examine limiting nutrients should not rely solely on this ratio, but instead couple it with bioassay studies at least initially in order to get more precise results.

Alternatively, the land cover in a watershed may provide information on the limiting nutrient status of a lake or reservoirs. Due to fertilizer runoff as well as over tilled soil that can no longer hold nutrients to the same degree as undisturbed soil, agricultural watersheds have been shown to have a high runoff rate of both N and P (Carpenter *et al*., 1998). This was supported in the current study based on the significant positive relationships between TN and TP and the percent of agriculture in the watershed. Similar positive relationships have been reported between agriculture land cover and a number of water quality variables including TN (Nielson *et al.*, 2012; Chen *et al.*, 2013), TP (Nielson *et al.*, 2012; Soranno *et al.*, 1996), chl-*a* (Nielson *et al.*, 2012), algal toxins (microcystin) (Beaver *et al.*, 2014), and sediment P release rates (Carter and Dzialowski, 2014) in surface waters. There were also significant differences in the percent land cover (agricultural and forest; there were no difference in the amount of developed land cover) of N and P limited reservoirs. If a watershed had more than ~40% forested land cover, it was likely to be primarily P limited, while if a watershed had more than ~25% agricultural land cover it was likely to be primarily N limited (Figure 8). This suggests that land cover could possibly be used as a predictive component to nutrient limitation in reservoirs. However, there are many factors that affect nutrient concentrations, and while understanding that watershed characteristic influence water quality and potentially nutrient limitation, it should not alone be the only factor considered. Furthermore, the relatively low relationship between TP and agriculture and the non-significant relationship between TP and forested land cover could be explained at least in part by the fact that sampling only occurred once from a single location on a single date in each reservoir. Water quality can vary both spatially and temporally within individual reservoirs (e.g. Nikolai and Dzialowski, 2014) and it likely that our samples were not representative of the entire reservoir. Further research should consider sampling multiple locations within a reservoir over the course of a season in order to test for better relationships between land cover, water quality, and nutrient limitation.

CHAPTER V

CONCLUSION

In conclusion, it was found that the majority of reservoirs were N and P co-limited. Fe never limited reservoirs alone, but it was found to be co-limiting with N and P together in a small number of reservoirs. While Fe did not cause a significant increase in bulk algal biomass, it may increase cyanobacteria and additional data are needed to better understand the relationships between Fe additions and cyanobacteria. There were significant relationships between some of the land cover types and water quality variables in the reservoirs, and reservoirs differed in percentage forest and agriculture depending on whether they were N or P limited. As such, it is possible that land cover may be an indicator of nutrient limitation at least in some reservoirs. While this study examined how three different land cover types related to total nutrients, it would be beneficial to include other land covers such as different forest types as well as different intensities of developed land to see if there is any additional relationships between land cover and water quality. The TN:TP ratio based on Guildford and Hecky (2000) were only able to predict the limiting nutrient in 56% of the reservoirs. Therefore, the TN:TP ratio should be used with caution in predicting nutrient limitation, and coupled with bioassay studies when possible. Combined, this study suggests that additional research should be conducted with Fe to better understand its role in cyanobacteria blooms, and to explore how other nutrients or land cover can affect nutrient limitation.

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