NUTRIENT LIMITATION OF ALGAE AND HETEROTROPHIC BACTERIA IN RESERVOIR ECOSYSTEMS: IMPLICATIONS FOR PELAGIC COMPETITION ALONG A TROPHIC GRADIENT

by

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With the acceptance and approval of this degree, I proclaim myself a Pokémon Master. This prestige can be accredited by the following people and agencies:

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LIST OF ABBREVIATIONS

Abbreviation	Description
ANOVA	Analysis of Variance
AND	Algal Nitrogen Demand
APD	Algal Production Demand
BGE	Bacterial Growth Efficiency
BND	Bacterial Nitrogen Demand
BOD	Biological Oxygen Demand
BPD	Bacterial Production Demand
BPr	Bacterial Production
BR	Bacterial Respiration
C	Carbon
Chl*a*	Chlorophyll *a*
DAPI	4', 6-diamidino-2-phenylindole
DIN	Dissolved Inorganic Nitrogen
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DON	Dissolved Organic Nitrogen
HDPE	High-Density Polyethylene
N	Nitrogen

NVSS	Non-Volatile Suspended Solids
P	Phosphorus
PAR	Photosynthetically Active Radiation
PPr	Primary Production
RR	Response Ratio
SRP	Soluble Reactive Phosphate
TCA	Trichloroacetic Acid
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
UV- Vis	Ultraviolet- Visible Light

ABSTRACT

In low productivity pelagic ecosystems with low concentrations of inorganic nutrients, bacteria have been shown to play a relatively greater role in C and nutrient cycling. The importance of bacteria is thought to decline as productivity and dissolved inorganic nutrients increases. Plankton ecologists have proposed several mechanisms which lead to this pattern, but it is generally thought that bacteria should exhibit a competitive advantage over algae for inorganic nutrients in unproductive systems with relatively high concentrations of dissolved inorganic C (DOC) and low concentrations of dissolved inorganic nutrients. However, there is a limited amount of data examining if the intensity of competition between algae and bacteria for inorganic nutrients varies with ecosystem productivity. My thesis focused on examining the potential for competition between heterotrophic bacteria and autotrophic algae across a productivity gradient in a group of 19 Texas and Ohio reservoirs. Across reservoirs, DOC:dissolved inorganic nutrient ratios decreased with increasing productivity, signifying a shift in the dominant forms of available nutrients for algae and bacteria along a trophic gradient. The N and P content of algal and bacterial cells (i.e., C:N and C:P) follow a similar pattern of increasing cellular nutrient content with increasing productivity. Concurrent nutrient limitation assays indicated that algae across reservoirs were primarily limited by N or P, whereas bacteria were most frequently primarily limited by P, less frequently by C, and almost never by N. The magnitude of nutrient limitation responses (i.e., calculated response ratios, RRs) were greater overall with P addition over N or C. Both algae and bacteria exhibited heightened response ratios to P than with N or C comparatively due to relatively lower concentrations of P (when compared to N and DOC) found within unproductive systems, thus an important limiting nutrient in these reservoirs studied.

CHAPTER 1

NUTRIENT LIMITATION OF ALGAE AND HETEROTROPHIC BACTERIA IN RESERVOIR ECOSYSTEMS: IMPLICATIONS FOR PELAGIC COMPETITION ALONG A TROPHIC GRADIENT

Introduction

Inland aquatic systems (i.e., lakes and reservoirs) receive water and materials from surrounding landscapes and function as sentinels for how landscapes and watersheds respond to short- and long-term changes in land use and climate (Williamson et al. 2009). Various environmental factors can affect the productivity of inland aquatic ecosystems, but cultural eutrophication in particular creates water quality, ecosystem health, and economic problems (Harper 1992; Dodds et al. 2009). Thus, for many inland water bodies, controlling the quantity and quality of nutrients and organic matter inputs from surrounding water- and airsheds is critical for management of osmotrophic algal and bacterial populations. Although heterotrophic bacteria and photoautotrophic algae in pelagic systems derive energy through different metabolic pathways, both bacteria and algae acquire and utilize inorganic nutrients such as nitrogen (N) and phosphorus (P) for growth and maintenance. Thus, bacteria and algae compete for inorganic nutrients (N and P) and the outcome of this interaction can determine the overall efficiency with which energy and nutrients are available to upper trophic level organisms (Cotner and Biddanda 2002).

Aquatic ecologists have hypothesized that the outcome of competitive interactions between heterotrophic bacteria and algae for inorganic nutrients is dependent upon overall ecosystem productivity (Cotner and Biddanda 2002). Under low inorganic nutrient and low productivity conditions, bacteria play a relatively greater overall role in

pelagic C and nutrient cycling. It has been hypothesized that there are several mechanisms which lead to this pattern (Cotner and Biddanda 2002). Bacterial cells have a high affinity for inorganic nutrients (Button 1986) and due to their generally smaller size bacteria have larger surface area:volume ratios, thereby facilitating more rapid uptake of inorganic nutrients than larger algal cells. In addition, small size and relatively simple structural complexity may confer bacteria an additional competitive advantage in unproductive systems because of lower energetic costs (Neidhardt et al. 1990). In addition, dissolved organic carbon (DOC) concentrations in unproductive and oligotrophic systems are typically greater than inorganic nutrients (Cotner and Biddanda 2002; Caston et al. 2009), and heterotrophic bacteria cells may additionally obtain a portion of their inorganic nutrient requirements from N or P bound into organic compounds (Graneli et al. 2004). Thus, the low relative importance of algae in C and nutrient dynamics under low inorganic nutrient and unproductive systems is due in part through a greater competitive advantage of bacteria under such conditions.

In contrast, as inorganic nutrient supply and productivity increases, the relative importance of bacteria should decline and the relative importance of algae in C and nutrient cycling should increase (Cotner and Biddanda 2002). Again, there are several potential mechanisms responsible for this pattern, but it is thought that as inorganic nutrient concentrations increase, algal populations are less constrained by competition with bacteria for inorganic nutrients (Cotner and Biddanda 2002). Although bacterial growth rates and growth efficiencies generally increase with trophic state (del Giorgio and Cole 1998; Biddanda et al. 2001), it has been hypothesized that in more productive systems, the overall DOC pool available to heterotrophic bacteria may be less labile

(Baines and Pace 1991; Gardner el al. 1996) or that a greater proportion of the pelagic C pool is lost to sedimentation before bacteria can process it (i.e., greater export production; Simon et al. 1992; Baines et al. 1994). In addition, protozoan grazing rates on bacteria increases with trophic state (Sanders et al. 1992), heterotrophic flagellates can select for larger and more rapidly growing bacterial cells (Sherr et al. 1992), and viral infection rates of bacteria also increase with system productivity (Weinbauer et al. 1993), potentially leading to depression of bacterial growth rates and biomass under more productive conditions.

Although ecologists have proposed a diversity of specific mechanisms that lead to the observed patterns in changing bacterial – algal importance along a trophic gradient (e.g., Biddanda and Cotner 2001; Caston et al. 2009), one of the most important underlying mechanisms influencing these patterns lies in the severity of competition between algal and bacterial populations for inorganic nutrients along a trophic gradient. Indeed, if competition for inorganic nutrients has a large influence on observed patterns, then it is critical to elucidate if algal and bacterial populations are concurrently limited by the same inorganic resources (N or P) and whether the severity of limitation for these resources changes with trophic state. Although the number of studies which have examined resource limitation in algae (typically, N and/or P) and bacteria (typically, N, P, and/or C) is extensive (e.g., Hecky and Kilham 1988; Davies et al. 2004; Graneli et al. 2004), few studies have simultaneously examined patterns of the severity of bacterio- and phytoplankton limitation across a large trophic gradient and determined the implications for these interactions for the trophic dynamics of lake and reservoir ecosystems (but see Chrzanowski and Grover 2001; Davies et al. 2004; Kolzau et al. 2014).

The overall objective of my study is to examine the potential for competition between heterotrophic bacteria and algae in a group of eleven Texas and eight Ohio reservoirs which represent an extensive productivity gradient. This study provides insight into the mechanisms leading to changes in the relative importance of the role of bacteria and algae along a trophic gradient. I examined several hypotheses about how the severity of limitation in algae (N or P limitation) heterotrophic bacteria (N, P, or C limitation) varied with a productivity gradient (Cotner and Biddanda 2002). I hypothesized that in lower productivity reservoirs, bacteria would compete more intensely with algae for inorganic nutrients. I predicted that DOC:inorganic nutrient ratios should be relatively high in these reservoirs and that both bacteria and algal populations would be strongly limited by inorganic nutrients (N or P). I further hypothesized that as trophic state and the availability of inorganic nutrients increased, the intensity of competition between bacteria and algae for inorganic nutrients would decrease, thereby allowing algal populations to have a greater role in pelagic nutrient dynamics. I predicted that DOC:inorganic nutrient ratios should decline with increasing trophic state and that the severity of inorganic nutrient limitation of bacterial and algal populations would decline. In addition, I predicted that the frequency and intensity of C limitation of bacterial communities would increase with trophic state (rather than inorganic N or P) and that the character of the ambient DOC would also change with increasing trophic state. Finally, I predicted that as reservoir trophic state increased the rate of bacterial production (BPr) and bacterial growth efficiencies (BGE) would increase, but that BGE would level off due to a decline in the lability of the DOC pool.

Methods

Data collection and field methods

Reservoirs from Texas and Ohio (USA) were sampled across the summer growing season in 2005, 2006, and 2014. Eight reservoirs distributed throughout Ohio were sampled a minimum of 2 times and a maximum of 9 times throughout summer 2005 (Table 1). Nine reservoirs in central Texas were sampled twice each in summer 2006 and six of these reservoirs were sampled again twice during summer 2014. Two of the Texas reservoirs were not resampled in 2006 (Table 1), but two additional reservoirs were sampled in 2014.

Detailed information on sampling and collection of data in the field for 2005 and 2006 are provided in Caston et al. (2009) and Texas reservoirs were sampled in 2014 with the same methods. Briefly, ambient conditions and water samples were taken at the deepest point in each reservoir, typically in close proximity to the dam. I measured depth profiles for temperature, dissolved oxygen, and conductivity using a Yellow Springs Instruments Model 85D (Texas) or Model 58 (Ohio). Water clarity depth was measured with a black and white Secchi disk. Photosynthetically active radiation (PAR) was measured with a Li-Cor Model 1935A meter and a 4π sensor. Using a weighted integrated tube, epilimnetic water was collected into opaque or brown high-density polyethylene (HDPE) Nalgene bottles and/or carboys and kept in coolers and transported to the lab.

Laboratory methods

Water samples were divided into subsamples in the lab for various analyses. Total phosphorus (TP) and total nitrogen (TN) were determined from unfiltered water samples. TP was measured as $PO^3_4^-$ after digestion with potassium persulfate using the molybdenum blue method (Wetzel and Likens 2000) on a Varian Cary 50 UV-Vis spectrophotometer (Texas) or on a Lachat QuickChem® FIA+ 8000 Series autoanalyzer (Ohio). TN was measured as NO_3^- after digestion with alkaline potassium persulfate and analyzed using second-derivative UV spectroscopy (Crumpton et al. 1992). Dissolved nutrients (PO_4^{3-}, NH_4^+, and NO_3^-) were estimated on samples filtered through a pre-ashed Pall A/E filter. PO_4^{3-} was estimated as soluble reactive phosphorus (SRP) using the molybdenum blue method (Texas) or on an autoanalyzer (Ohio). NH_4^+ was analyzed using the phenate method (Wetzel and Likens 2000). NO_3^- was determined with second-derivative UV spectroscopy (Crumpton et al. 1992) in Texas and the cadmium reduction method (Wetzel and Likens 2000) in Ohio. Dissolved organic carbon (DOC) samples were filtered through pre-ashed Whatman GF/F filters, and the filtrate was analyzed on a Shimadzu TOC-V_{CSH} total organic carbon analyzer. Dissolved inorganic nitrogen (DIN) was calculated by adding NH_4^+ and NO_3^-. In 2014 (but not 2005 and 2006), samples for water color (a potential surrogate measure for DOC quality) was ascertained by filtering water through a pre-ashed Whatman GF/F filter and measuring UV absorbance at 440 nm (Cuthbert and del Giorgio 1992). Non-volatile suspended solids (NVSS) was ascertained with water filtered with pre-combusted and pre-weighed A/E filters. Filters were dried at ~50℃ for 48 h and weighed again to determine total suspended solids (TSS). Filters were combusted subsequently at 500℃ for 4 h and re-weighed to determine NVSS.

Particulate C, N, and P in bacteria were measured in particles <1 µm, and algal C, N, and P was measured in particles >1 µm (Caston et al. 2009). Particulate C and N was filtered onto pre-ashed Whatman GF/F filters, dried at 60°C for 48 h, and analyzed using a CE Elantech Carbon–Nitrogen analyzer (Texas) or a Perkin Elmer Carbon-Hydrogen-Nitrogen analyzer (Ohio). Particulate P was determined with water filtered onto pre-ashed Whatman GF/F filters, digested with concentrated HCl and measured as PO_4^{3-} as above.

Phytoplankton biomass was estimated as Chl*a* by filtering water onto Pall A/ E glass-fiber filters. Filters were frozen until analysis and Chl*a* was extracted with HPLC-grade acetone for a minimum of 4 h in the dark, and measured using a Turner Designs Trilogy® fluorometer (Texas) or a Turner TD-700 fluorometer (Ohio). In 2014 only, duplicate bacteria samples from each reservoir were preserved with filtered (< 0.2 µm) formalin and kept in the dark at 4°C until bacteria cell density was determined by filtering samples onto 0.2-µm black Nucleopore membrane filters, stained with 4'6-dianidino-2-phenylindole (DAPI), and cells were counted at 100x magnification. For each sample, thirty fields of view (grid area= 1.0×10^{-4} mm^2) were counted (Kemp et al. 1993).

Nutrient limitation assays

In order to determine the nutrient limitation status of algae and bacteria, nutrient limitation assays were conducted with water brought back to the lab. Here, I define nutrient limitation as a change in the per unit growth rate of an algal assemblage following the addition of nutrients added in surplus (Osenberg and Mittelbach 1996). Assays measure the degree of limitation of both bacteria and algae to individual nutrients

and in combination and nutrient limitation in the context of this study was assessed by evaluating the change in biomass (for algae) or productivity (bacteria) after the addition of potentially limiting nutrients in excess (Elser et al. 2007).

Algal nutrient limitation assays were conducted using methods described in Vanni et al. (2006). Briefly, water was first passed through an 80-µm Nitex screen to remove zooplankton grazers and the filtered sample was divided into duplicate or triplicate flasks of four assay treatments: Control (no nutrients), +N (50 µmol/L N added as NH_4NO_3), +P (2.5 µmol/L P added as $NaH_2PO_4*H_2O$), and +N+P. Flasks were incubated at epilimnetic temperature at ~200 µmol PAR m^2 sec, swirled after 24 h incubation to resuspend algae, and assays were terminated after 48 h by filtering contents of each flask and analyzing for Chl*a* concentration using above methods.

For bacteria nutrient limitation assays, water was passed through a 1-µm Nuclepore® membrane filter to exclude bactiviores. Assays included eight treatments: Control (no nutrients), +N (50 µmol/L N added as NH_4NO_3), +P (2.5 µmol/L P added as $NaH_2PO_4*H_2O$), +C (259 µmol/L C added as glucose), +N+P, +N+C, +C+P, and +N+P+C. Treatments were performed in duplicate or triplicate. Bacterial assays were incubated in the dark at lake temperature for 48 h and flasks were swirled after 24 h. At the end of the incubation, bacterial production (^{3}H-leucine incorporation method; see *Production Estimates* below) was measured to quantify responses.

I assessed nutrient limitation of algal and bacterial populations in each reservoir on each sampling date using two methods. First, I determined the nutrient (N or P for algae and N, P or C for bacteria) which elicited the largest response after the incubation period using the actual Chl*a* concentrations or bacterial production rates. Because assay

experimental designs were cross-classified (e.g., Control, +N only, +P only, and +N+P for algal assays), I performed a two-way (for algae) or three-way (for bacteria) ANOVA to determine the effects of each nutrient amendment. If the overall ANOVA detected a significant positive main effect of a nutrient amendment (N, P, or C), then I considered algae or bacteria to be limited by that nutrient. However, if multiple single addition nutrients elicited a significant positive effect on algae or bacteria, I then compared these positive responses using a one-way ANOVA in order to determine the primary positive limiting nutrient for each reservoir and date. The single nutrient that elicited the significantly greatest enhancement was considered to be the primary limiting nutrient for algal or bacterial populations. Thus, for each bioassay conducted, I could classify the outcome as indicating no limitation of any of the added nutrients, primarily P-limited, primarily N-limited, or primarily C- limited (for bacterial only).

Although the above "vote counting" method provides information on the nutrient that is primarily limiting algal or bacterial populations in a reservoir at a specific point in time, it does not provide information on the severity or degree of limitation. In order to obtain a quantitative estimate of the severity of limitation, I calculated a response ratio (Downing et al. 1999; Elser et al. 2007) in which the response ratio (*RR*) is the degree of growth due to nutrient limitation under incubation conditions for each treatment

$$RR = \ln\left(\frac{N_E}{N_C}\right)$$

where, N_E is the biomass or productivity in treatments enriched with added N, P, or C concentrations (or combinations of multiple nutrients), N_C is the biomass or productivity in the control treatments which receive no additional nutrients. The magnitude of the value of *RR* is related to extent or severity of nutrient limitation with each type of nutrient

and their synergistic effects on growth of bacterial or algal cells over several days (Downing et al. 1999). The response ratio is a commonly used limitation metric and allows for comparisons across systems and the analysis of change relative to the control is more meaningful than standardized absolute differences between means (Elser et al. 2007). For these analyses, I did not include the combined nutrient treatments (e.g., +N+P, +N+P+C), which would have allowed for the determination of potential co-limitation and serial limitation of nutrients (Harpole et al. 2011). I elected to not assess co-limitation or serial limitation in this study because I was primarily interested in the nutrient that was primarily limiting bacterial and algal communities and assessment of these types of limitation were largely outside the scope of this thesis. However, I will assess these types of co-limitation and serial limitation issues in algal and bacterial communities in a manuscript.

Production Estimates

For all reservoirs across all years, bacterial production (BPr) was measured using the ^{3}H-leucine microcentrifuge method (see Caston et al. 2009 for detailed methods). For each reservoir, four "live" and two "dead" 1.5 ml water samples were incubated with ^{3}H-leucine for ~ 1 h in microcentrifuge tubes. BPr estimates were calculated as in Caston et al. (2009).

In 2014 only, I estimated bacterial respiration (BR) through the use of biological chemical demand (BOD) incubations (Roland et al. 1999; Williams and del Giorgio 2005). For each reservoir five acid-washed and cleaned 60-mL Whatman BOD bottles with glass stoppers were filled with water. Two bottles were immediately sacrificed and used to estimate the initial DO concentrations (Day 0). The remaining three bottles for

each site were incubated in the dark at *in situ* reservoir temperature for 48 h. DO was measured using a modified spectrophotometric Winkler method (Roland et al. 1999). Hourly O_2 consumption (mg O_2 L^{-1} h^{-1}) was determined by calculating the difference between the initial and Day 2 DO concentrations. O_2 consumption rates were converted to C respired (mg C L^{-1} h^{-1}) based upon a respiratory quotient of 1. Bacterial growth efficiency (BGE) for each reservoir on each date was estimated with BPr (see above) and BR values using the equation BPr / [BPr+BR] (del Giorgio and Cole 1998).

Primary production (PPr) was estimated for Ohio (2005) and Texas (2006) reservoirs using the ^{14}C method and details are presented in Caston et al. (2009). PPr rates were estimated using biomass-corrected Chl*a* concentrations and depth-specific PAR measurements from the field in addition to the estimation of the following parameters: slope of the photosynthesis-irradiance curve at low PAR levels (α^B) and the photosynthetic rate at optimal PAR levels (P^B_M) (Caston et al. 2009).

In order to further assess the potential for algae and bacteria for inorganic nutrients across a productivity gradient, I estimated bacterial and algal P demand (BPD and APD, respectively) and N demand (BND and AND, respectively) using production estimates (PPr and BPr) and the C:P and C:N ratios of bacteria and algae on each sampling date in 2005 and 2006. Estimated demand was calculated by multiplying the hourly C production rates of bacteria and algae (in moles) by the C:P or C:N (molar) of each planktonic fraction. This method of estimating nutrient demand assumes that as bacterial or algal communities are producing new C biomass at a point in time, the C:P and C:N of these communities indicate that they must be incorporating P and N at a rate to reflect the measured sestonic ratios.

Data analysis

SPSS (version 20) software was used for all analyses. Ordinary least squares linear regressions were used to test the hypotheses and describe the strength of relationships between nutrient ratios (DOC:DIN, DOC:TN, DOC:SRP, and DOC:TP), algal and bacterial P and N demand, and the productivity gradient represented by the reservoirs in this study. I utilized Chl*a* as my measure of trophic state because is it often used to express trophic state and Cotner and Biddanda (2002) use it as their measure of trophic state. As in previous studies examining nutrient dynamics and algae and bacteria across a trophic gradient, many of the relationships were best described as a power curve (Cotner and Biddanda 2002; Caston et al. 2009), so all data were log-transformed all before analysis and I expressed the relationship between variables with a linear regression of the form $\log(y) = b \log(x) + c$, where y is the dependent variable, x is the independent variable, b is the slope, and c is the y-intercept. Regression equations can be converted to power curves of the form $y = (d\, x^b)$ where d is the antilog of c. For all figures, I present the data in untransformed format so that the magnitude and nature of relationships are apparent.

When examining relationships between algal and bacterial *RR*s and ambient nutrient concentrations and the stoichiometry of algae and bacteria, I did not explicitly attempt to fit data into regression models, but rather explored the nature of the relationships between these variables by examining the distribution of data along these gradients. Finally, when examining trends in BPr and BGE across the reservoir trophic gradient and DOC_{Color}, I used ordinary least squares linear regression. For all analyses, statistical significance was inferred at $\alpha = 0.05$.

Results

Across all reservoirs, Ohio reservoirs were typically shallower and had smaller surface areas than Texas reservoirs (Table 1). Although there was some variation in limnological parameters, water chemistry, and production values between sampling dates for each reservoir, this variation was typically smaller than the variation observed between reservoirs (Table 2). Across all reservoirs and dates, the systems examined in this study represented a substantial trophic gradient (1.30 – 73.81 µg Chl*a* L^{-1}). In general, Ohio reservoirs tended to have greater nutrient concentrations, algal biomass, and production rates than Texas reservoirs, but in combination these two sets of reservoirs represent one larger continuous pattern because Texas and Ohio reservoirs with similar trophic status (i.e., Chl*a* concentration) also have similar nutrient concentrations and productivities (Caston et al. 2009).

Across reservoirs, the mean DOC:inorganic nutrient ratios decreased with increasing Chl*a* (Fig. 1). Across all reservoirs, DOC concentrations were greater than dissolved (DIN and SRP) and total (TN and TP) nutrient pools, as indicated by the DOC:nutrient ratios being >1 (Fig. 1). As predicted, ratios of DOC:DIN (Fig. 1A), DOC:TN (Fig. 1B), and DOC:TP (Fig. 1D) significantly declines with increasing Chl*a*.

When I examined nutrient limitation assay results and determined the primary limiting nutrient (i.e., vote counting results), I found that P was an important limiting nutrient for both algae and bacteria (Table 3). For algal communities across all reservoirs, 82% of all assays indicated that algae exhibited a significant positive response to P and 50% of all assays indicated that P was the primary limiting nutrient. Nitrogen

also frequently elicited a significant positive response in algal communities across all assays (55% of assays) and 41% of all assays indicated that N was the primary limiting nutrient. Thus, primary P limitation of algae was only slightly more frequent than N. Out of all conducted assays, 7.58% found no significant response, either positive or negative, in comparison to the control.

For bacteria, P was clearly more important than either of the two other nutrients in terms of primary limitation. Across all experiments, 85% of showed a significant positive response to P, with 72 % of assays indicating P was the primary limiting nutrient (Table 3). Addition of N less frequently elicited a significant positive response (51% of assays), but only 3% of assays found that N was the primary limiting nutrient. Addition of C elicited a significant increase in 71% of assays, but only 21% of experiments indicated C was the primary limiting nutrient. 4% of all the assays conducted resulted in no significant response to any single nutrient addition (+N, +P, or +C).

Examining algal limitation in the context of response ratios (*RR*), I found that the magnitude of the response of algal communities to added nutrients (both positive and negative) declined in systems with higher nutrient concentrations (Fig. 2). RR_N of algae across all reservoirs and all sampling dates decreased with increasing concentrations of both DIN and TN concentrations (Fig. 2A and B). Responses to N additions ranged from -1.01 to 0.987, which translated to a 105 - 624% change relative to control assays. A closer examination of responses indicated that most of the higher magnitude responses (>88%) occurred when TN and DIN were $\leq$100 µM L^{-1}. Similarly, the magnitude of algal RR_P values decreased with increasing TP and SRP concentrations (Fig. 2C and D). In general, RR_P values were higher than algal RR_N values (values were up to 1.83). In

addition, most of the high magnitude positive and negative RR_P values occurred when SRP and TP <0.25 and 3 µM L^{-1}, respectively.

Bacterial *RR* values across ambient nutrient gradients in all reservoirs showed a similar pattern to those observed in algae, but bacterial RR_P values were almost exclusively positive, regardless of the P concentration (Fig. 3A - H). Again the magnitude of *RR* values generally declined with increasing DIN, TN, SRP, and TP. In addition, the magnitude of bacterial RR_P responses declined with increasing epilimnetic DOC:SRP. Bacterial RR_C responses exhibited little clear directional relationship with the ambient DOC concentration or DOC_{color} (Fig. 3G and H).

In contrast to the patterns observed in algal *RR*s to ambient nutrient concentrations, algal RR_N and RR_P responses were much less consistent when examined over the range of the nutrient ratios of the algal fraction of the plankton (Fig. 4). Algal RR_N values did not clearly or consistently vary with algal N:P and C:N ratios (Fig. 4A and B). Algal RR_P values similarly exhibited little consistent response to algal N:P and C:P ratios (Fig. 4C and D). Bacterial RR_N were not consistently related to increasing bacterial N:P or C:N (Fig. 5A and B), but a clear majority of RR_P ratios were positive, regardless of bacterial N:P and C:P ratios (Fig. 5C and D). Bacterial RR_C values were not clearly related to bacterial C:N and C:P (Fig. 5E and F).

Bacterial metabolism was clearly related to the trophic gradient present in this study (Fig. 6), with BPr (r^2= 0.76, p< 0.0001) and BR (r^2= 0.81, p= 0.0023) significantly increasing with Chl*a* (Fig. 6A and B). Bacteria cell density significantly increased with increasing Chl*a* (r^2= 0.47, p= 0.003). However, contrary to my hypothesis, BGE was not related to Chl*a* (r^2= 0.04, p= 0.62) or DOC_{Color} (r^2= 0.34, p= 0.13) (Fig 6C - D).

When I examined the ratio of bacterial P demand to algal P demand (BPD:APD) as a function of the productivity gradient across reservoirs, BPD:APD declined significantly with increasing Chl*a* (r^2= 0.74, p< 0.0001). In addition, in the lower productivity systems, BPD:APD ratios were relatively high, exceeding a value of 1.0 in two of the more oligotrophic reservoirs (Fig. 7A), indicating that bacterial demand for P was greater than that of algae. However, this relationship quickly declined with increasing trophic status and BPD was a very small fraction of APD (BPD:APD= 0.01-0.176). The ratio of BND:AND also declined with increasing Chl*a* (r^2= 0.75, p< 0.0001), but the value never exceeded >0.07 in the most oligotrophic systems, indicating that algal N demand was always greater than bacterial N demand, regardless of reservoir trophic status (Fig. 7B).

Discussion

The results of the present study generally support the hypotheses in that DOC concentrations in more oligotrophic systems were relatively high and inorganic nutrients (P and N) were low (indicated by high DOC:inorganic nutrient ratios) and that DOC:inorganic nutrient ratios decreased with increasing trophic status. In the set of reservoirs examined by this study, both inorganic nutrients and DOC increased with trophic status, but inorganic nutrients (DIN and PO_4^{3-}) increased with Chl*a* concentration at a faster rate than DOC, resulting in declining DOC:inorganic nutrient ratios. It has been hypothesized that as ambient N and P concentrations and overall system production increases, the severity of limitation of both algae and bacteria communities by inorganic

nutrients should decline, resulting in a decrease in the intensity of competition of algae and bacteria for inorganic nutrients. Indeed, in the present study I observed that both algae and bacteria generally exhibited greater *RR* responses in lower ambient nutrient systems, indicating greater inorganic nutrient limitation in less productive systems and a greater potential for competition between algae and bacteria for these resources. This result suggests that both algal and bacterial communities were less deficient in inorganic nutrients and there was therefore less competition for access to these resources.

In the present study, I found that the primary limiting nutrient for algal communities was split fairly evenly between N and P. Algae exhibited lower *RRs* with increasing productivity, showing P to be the primary limiting nutrient still within these reservoirs. Historically, ecologists have identified P as the primary limiting nutrient in many inland systems (Schindler 1977; Hecky and Kilham 1988; Elser et al. 2007), but it is not uncommon N addition (or co-limitation by N and P together) to also occur in pelagic systems (Elser et al. 1990; Maberly et al. 2002; Dzialowski et al. 2005; Paerl et al. 2014). There is a growing body of literature that clearly indicates that N can be an important limiting nutrient in a diversity of freshwater ecosystems (Wurtsbaugh et al. 1985; Elser et al. 2007). The present study clearly supports this growing body of literature that, while P is undoubtedly important for algal communities, N can certainly be a critical primary limiting nutrient as well.

In contrast to algal communities, bacteria in the present study were most frequently primarily limited by P and N was very rarely primarily limiting. Previous studies indicate that bacteria compete strongly with algae for inorganic P (Currie and Kalff 1984; Danger et al. 2007), often account for a substantial portion of the P

sequestered in plankton communities, and can be responsible for a majority of the epilimnetic P uptake (Mazumder et al. 1988; Davies et al. 2004; Nowlin et al. 2005). Thus, the results of this study and a substantial body of literature clearly indicate that P is of primary importance for bacterial communities across a diversity of freshwater systems and that P-limitation occurs much more frequently than N limitation. The lack of primary N-limitation by bacteria in the present study may be due to the fact that N-containing compounds are often associated with and bound into organic C (Vitousek et al. 2002; Knicker 2004). It has been suggested that bacteria may meet a portion of their N demands through the uptake of N-containing DOC (Graneli et al. 2004), however, I did not assess concentrations of dissolved organic N (DON) or the potential use of DON compounds by bacterial communities in the present study. With high ambient N:P ratios found in the tested oligotrophic systems, this indicates a key mechanism from the bottom-up control perspective on production based upon the competition for inorganic nutrients (Graneli et al. 2004; Caston et al. 2009).

Results from my study in general do not support the hypothesis that bacterial C limitation will become more pronounced as reservoir productivity increases. In the systems examined by the current study, DOC:inorganic nutrient ratios declined with increasing trophic status, but bacterial communities did not show an increase in the frequency of C-limitation or the intensity of *RRs* with increasing trophic state. Across systems examined by my study, even the highest trophic state systems exhibited DOC:DIN and DOC:SRP values as high 25:1 and 2500:1, respectively, suggesting that DOC concentrations may not have been low enough for DOC to become limiting over inorganic nutrients, P specifically. In addition, I primarily examined how bacterial C-

limitation was related to the concentration of the "bulk" DOC pool, but using bulk DOC to examine the availability of C to bacteria presents substantial limitations (Caston et al. 2009). Indeed, the overall pool of DOC is composed of a variety of C sources that vary in quality and lability and bulk DOC measurements do not provide any information about the composition of the DOC pool. Furthermore, it is possible that the DOC pool in the reservoirs examined in this study was dominated by autochthonously-derived (algal) C and these exudates were in excess of bacterial community C demand (Bertilsson and Jones 2003). This scenario may be even more likely in the higher trophic state reservoirs which have lower DOC:PO_4^{3-} ratios, but greater algal biomass, and potentially greater release rates of labile DOC.

In the present study, I used DOC color (absorbance at 440 nm) as a potential surrogate for the general quality of DOC pool and as an indicator of the intensity of the connection between the watershed and the reservoir, but not as a direct measurement of color (Cuthbert and del Giorgio 1992; Pace and Cole 2002). As stated previously, I did not determine the composition of the DOC in relation to terrestrial and/or autochthonous C sources. Interestingly, in the present study DOC_{Color} significantly increased with trophic state, indicating that as algae become more concentrated, the DOC character changes substantially. Clearly, further work is needed to investigate how DOC concentration, DOC_{Color}, and algal biomass are related to each other in my study reservoirs. In addition, metrics such as DOC_{Color} may not be good predictors of overall DOC quality in many systems. Other metrics, such as specific UV absorbance at 254nm ($SUVA_{254}$) (Weishaar et al. 2003) or by XAD-resin fractionation (Hanley et al. 2013) are likely to be more useful or insightful for examining the role of DOC quality in bacterial

C- limitation. I hypothesized that BPr and BGE would increase with reservoir trophic state, but BGE would either plateau at a given trophic state or begin to decline due to a decline in the availability of labile DOC (Biddanda and Benner 1997; del Giorgio et al. 1998; Cotner and Biddanda 2002). My results indicate that BGE exhibited no relationship with trophic state (Chl*a*). In their review of BGE, del Giorgio et al. (1998) found that BGE values along a trophic gradient exhibit a large amount of variation, resulting in broad trends that eventually lead to a decoupling between BP and BR in higher trophic status systems. When I compare our range of estimates of reservoir BGE (range = 0.11 – 0.26) fall within the range of BGE estimates from lakes reported by del Giorgio et al. (1998) (range = 0.04-0.66), but I found that BGE did not vary with reservoir trophic state. The lack of a relationship between BGE and trophic state suggests that BGE may be more related to other factors that were not assessed (e.g., DOC composition and DOC quality). Del Giorgio et al. (1998) suggest the BGE relationship they observed could be due to several factors, including covariance of BGE and bacterial growth rates, cell-specific production, but I did not assess these relationships in the present study.

In the present study, I found that bacteria demand for both P was equivalent or higher than that of algae in several of the less productive systems examined (i.e., BPD:APD). Previous work on these reservoirs indicate that the ratio of BPr:PPr declined with increasing Chl*a*, but that BPr never exceeded PPr in any system (Caston et al. 2009). However, in the present study, bacterial C:P ratios ranged substantially lower than algal C:P, indicating that bacteria were more P rich than algae in all systems. When bacterial P demand estimates are examined in the light of the limitation results, the conclusion is that

bacteria are likely constraining algal communities through competition for P specifically in lower trophic state systems. Thus, the hypothesized mechanism of bacteria constraining algal production needs to be revised to specifically address P and not N.

Although the results of my study clearly indicate that algal production and biomass is restrained in lower trophic state systems through competition with bacteria for inorganic P sources, I did not address the potential mechanisms which are hypothesized to reduce the relative importance of bacteria in more productive systems. Indeed, in lower trophic state systems, it seems that bottom-up forces (competition and access to inorganic nutrients) are primarily important in determining the relative roles and bacteria and algae. However, in more productive systems, top-down forces, such as protozoan grazing on bacteria and viral infection may have a larger role (Sanders et al. 1992; Weinbauer et al 1993). The present study found that bottom up forces, such as C-limitation of bacteria did not occur in more productive systems, thus I hypothesize that top-down forces are likely to be more influential in determining the relative importance of bacteria in these systems. However, there is a need to understand how these potential mechanisms vary with trophic state to regulate the role bacteria in nutrient and C dynamics.

Table 1. Characteristic morphometry summary for studied reservoirs in central Texas (2006, 2014) and Ohio (2005), U.S.A. Location, years water samples collected (Year(s) Sampled), number of times sampled (Sampling Total), surface area measured near-dam over growing season (S.A.), Secchi depth (Secchi), mixed layer depth (z_{mix}), Ohio and Texas 2006 data measurements collected from Caston et al. (2009) and Ground and Groeger (1994). Data for each variable was averaged for the two or more sampling events.

Reservoir	Lat, Long (°N) , (°W)	Year(s) Sampled	Sampling Total	S.A. (km^2)	Secchi (m)	z_{mix} (m)
Texas						
Bastrop	30.16, 97.29	2006	2	3.7	2.00	6.00
Buchanan	30.75, 98.42	2006	2	93.3	1.93	7.50
Medina	29.54, 98.93	2006	2	22.6	3.23	8.5
Belton	31.10, 97.48	2014	2	50.6	2.9	10.00
Georgetown	30.66, 97.73	2014	2	5.3	1.88	4.25
Dunlap	29.65, 98.07	2006, 2014	2	1.7	0.75, 0.74	2.0, 1.50
Granger	30.7, 97.32	2006, 2014	4	17.8	0.48, 0.7	6.75, 7.00
Inks	30.73, 98.37	2006, 2014	4	3.2	1.65, 1.55	2.5, 4.25
LBJ	30.55, 98.34	2006, 2014	4	25.8	1.58, 1.73	4.0, 3.0
Canyon	29.88, 98.26	2006, 2014	4	33.3	4.3, 3.2	10.0, 9.0
Stillhouse Hollow	31.02, 97.53	2006, 2014	4	26	4.60, 3.75	8.5, 10.0
Ohio						
Acton	39.56, 84.74	2005	9	2.6	0.76	2.6
Berlin	41.05, 81.00	2005	2	4.5	0.96	4.5
Burr Oak	39.54, 82.06	2005	8	3.0	1.72	3.0
Delaware	40.36, 83.07	2005	2	4.0	0.65	4.0
Dillon	39.99, 82.08	2005	2	3.5	1.01	3.5
Hoover	40.11, 82.88	2005	2	4.3	0.95	4.3
Pleasant Hill	40.62, 82.32	2005	7	4.1	0.92	4.1
Tappan	40.35, 81.23	2005	2	4.8	0.83	4.8

Table 2. Water quality data from the 19 studied reservoirs in Ohio (2005) and central Texas, U.S.A (2006, 2014). Chlorophyll *a* (Chl*a*), total phosphorus (TP), total nitrogen (TN), dissolved inorganic nitrogen (DIN, dissolved organic carbon (DOC), nitrate (NO_3), ammonium (NH_4), soluble reactive phosphorus, and non-volatile suspended solids (NVSS). Ohio and Texas 2006 data measurements collected from Caston et al. (2009) and Ground and Groeger (1994). Data for each variable was averaged for the two or more sampling events. All units are in µ M L^{-1} except for chl*a* (µ g L^{-1}) and NVSS (mg L^{-1}).

Reservoir	Chl*a*	TP	TN	DIN	DOC	NO_3	NH_4	SRP	NVSS
Texas									
Bastrop	8.34	0.93	58.49	7.93	957	5.94	1.98	0.05	0.01
Buchanan	7.53	0.4	26.97	3.09	403	2.25	0.84	0.04	0.07
Medina	1.51	0.21	12.25	2.52	240	1.99	0.53	0.04	0.80
Belton	5.07	1.18	29.59	10.04	254	4.06	5.97	0.06	1.14
Georgetown	5.84	0.34	23.76	7.13	226	3.18	3.95	0.06	1.02
Dunlap	16.46, 14.71	1.13, 1.62	76.76, 63.78	64.16, 45.62	255, 202	60.02, 39.61	4.14, 6.01	0.11, 0.20	3.31, 6.54
Granger	15.07, 17.23	1.09, 1.33	33.82, 37.83	4.38, 10.82	326, 235	2.77, 5.93	1.61, 4.89	0.18, 0.08	11.53, 8.31
Inks	12.27, 19.44	0.94, 0.86	29.75, 40.56	3.62, 9.54	411, 343	2.18, 5.67	1.43, 3.87	0.06, 0.10	0.12, 0.76
LBJ	11.23, 22.73	0.51, 0.46	28.96, 40.00	3.78, 9.35	424, 302	2.44, 5.32	1.33, 4.03	0.04, 0.05	0.21, 0.49
Canyon	1.30, 1.48	0.16, 0.17	11.54, 16.25	0.99, 7.38	274, 179	0.82, 3.98	0.17, 3.40	0.04, 0.05	0.39, 0.46
Stillhouse Hollow	1.50, 2.29	0.21, 0.21	16.25, 22.65	2.32, 8.10	347, 237	1.79, 4.31	0.52, 3.79	0.05, 0.09	0.03, 0.16
Ohio									
Acton	73.64	2.41	334.18	271.47	229.8	265.26	6.21	0.18	2.12
Berlin	15.34	0.84	32.96	3.94	356.2	0.56	3.38	0.05	4.62
Burr Oak	15.09	0.58	25.43	3.31	230.8	0.62	2.68	0.10	1.21
Delaware	73.81	2.64	135.01	60.93	345.7	53.53	7.40	0.11	6.50
Dillon	30.11	10.10	81.80	28.74	136.3	8.33	20.41	3.88	2.93
Hoover	20.57	1.05	54.10	14.18	342.7	8.83	5.35	0.06	1.01
Pleasant Hill	49.09	1.83	68.23	17.78	147.4	13.75	4.03	0.17	1.54
Tappan	46.65	1.16	46.59	2.53	192.3	0.21	2.31	0.09	0.13

Table 3. Nutrient limitation assay frequencies for primary limiting nutrients in algal and bacterial samples taken from eight reservoirs in 2014. The most significant *RR* from a single nutrient addition is the most limiting to an organism frequencies of the primary limiting nutrient within all sampling periods during 2005-2014 from each reservoir, Percentage of limitation assays that resulted in a primary limitation for each single nutrient addition for algae *n*= 66 and bacteria *n*= 68 expressed as a percentage.

Organism	Primary Limiting Nutrient	Frequency (*n*)	Primary Limitation (%)
Algae	N	27	41.0
	P	33	50.0
Bacteria	N	2	2.94
	P	49	72.05
	C	14	20.59

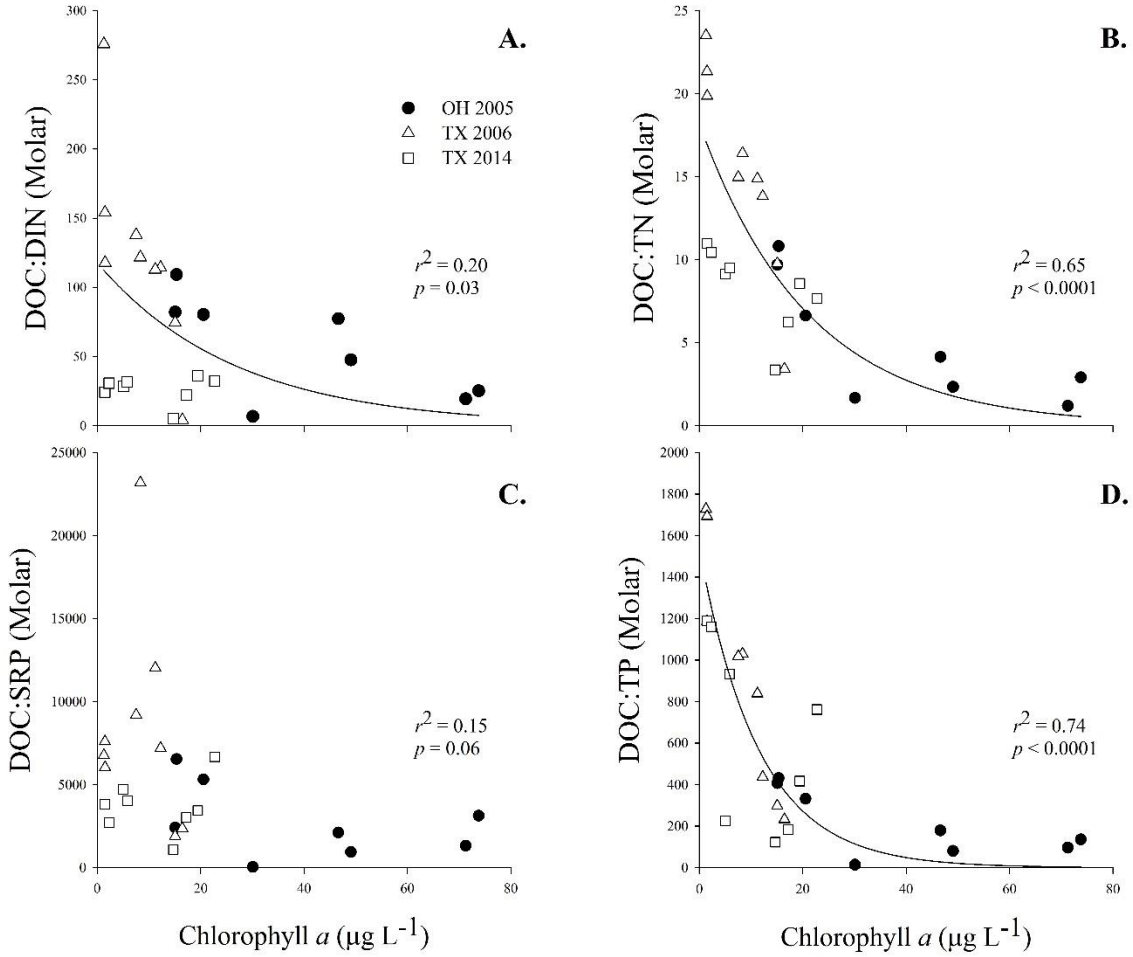

Figure 1. Relationship of chlorophyll *a* as a function of dissolved and total inorganic nutrients. (**A**) dissolved organic carbon to dissolved inorganic nitrogen (DOC:DIN), (**B**) dissolved organic carbon to total nitrogen (DOC:TN), (**C**) dissolved organic carbon to soluble reactive phosphate (DOC:SRP), and (**D**) dissolved organic carbon to total phosphorus (DOC:TP) within central Texas and Ohio reservoirs (*n*= 25). Exponential decay regression lines indicate significant relationships. Inset legend in Figure 1A is the same for all figures A-D.

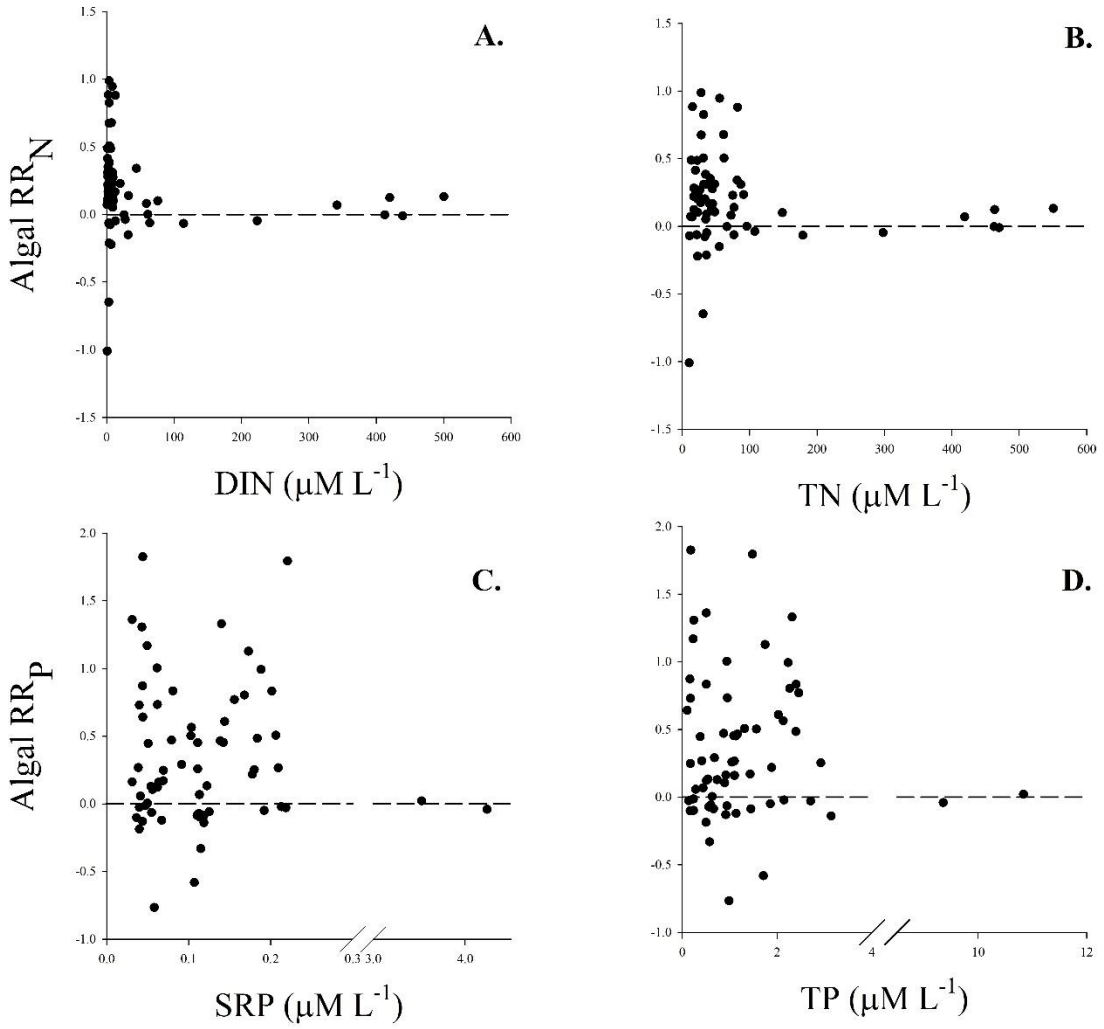

Figure 2. Comparisons of ambient water stoichiometry and phytoplankton response ratios. (**A**) Response ratios of nitrogen (N) for algae versus dissolved inorganic carbon (DIN) and (**B**) total nitrogen (TN) Molar values. **(C**) Algal response ratios for soluble reactive phosphorus (SRP) and (**D**) total phosphorus (TP) Molar values. All data points consist of each individual sampling period for each date from Ohio and central Texas, U.S.A reservoirs.

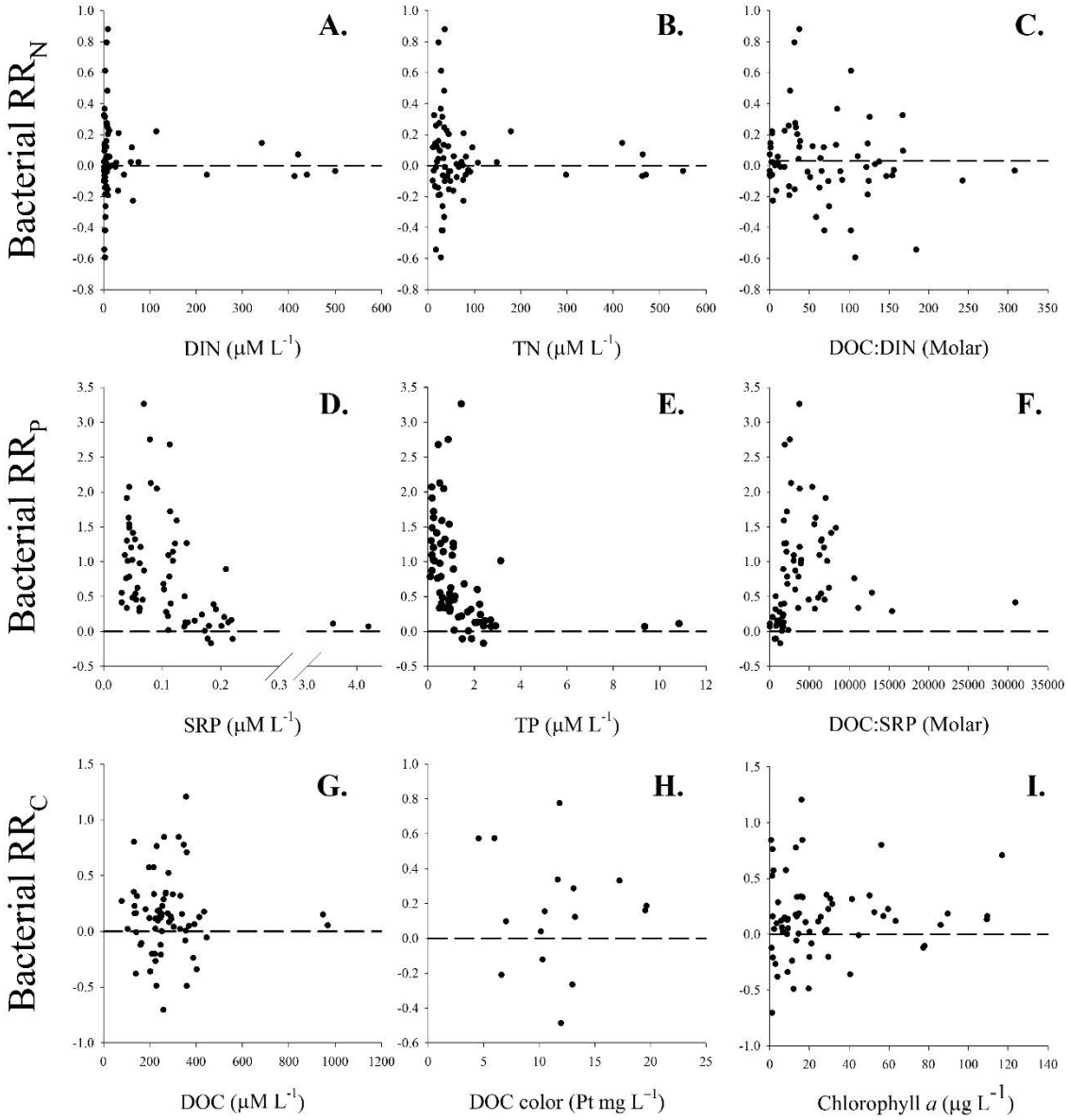

Figure 3. Water stoichiometry and heterotrophic bacteria response ratios. (**A**) Response ratios of nitrogen (N) for algae versus dissolved inorganic nitrogen (DIN), (**B**) total nitrogen (TN), (**C**) and dissolved organic carbon to dissolved inorganic carbon ratios (DOC:DIN). (**D**) Algal response ratios for phosphorus (P) versus soluble reactive phosphorus (SRP), (**E**) total phosphorus (TP), and (**F**) dissolved organic carbon: soluble reactive phosphorus (DOC:SRP) molar values. (**G**) Algal response ratios for carbon (C) versus dissolved organic carbon (DOC), (**H**) DOC color, and (**I**) chlorophyll *a* values. All data points consist of each individual sampling period for each date from Ohio and central Texas, U.S.A reservoirs.

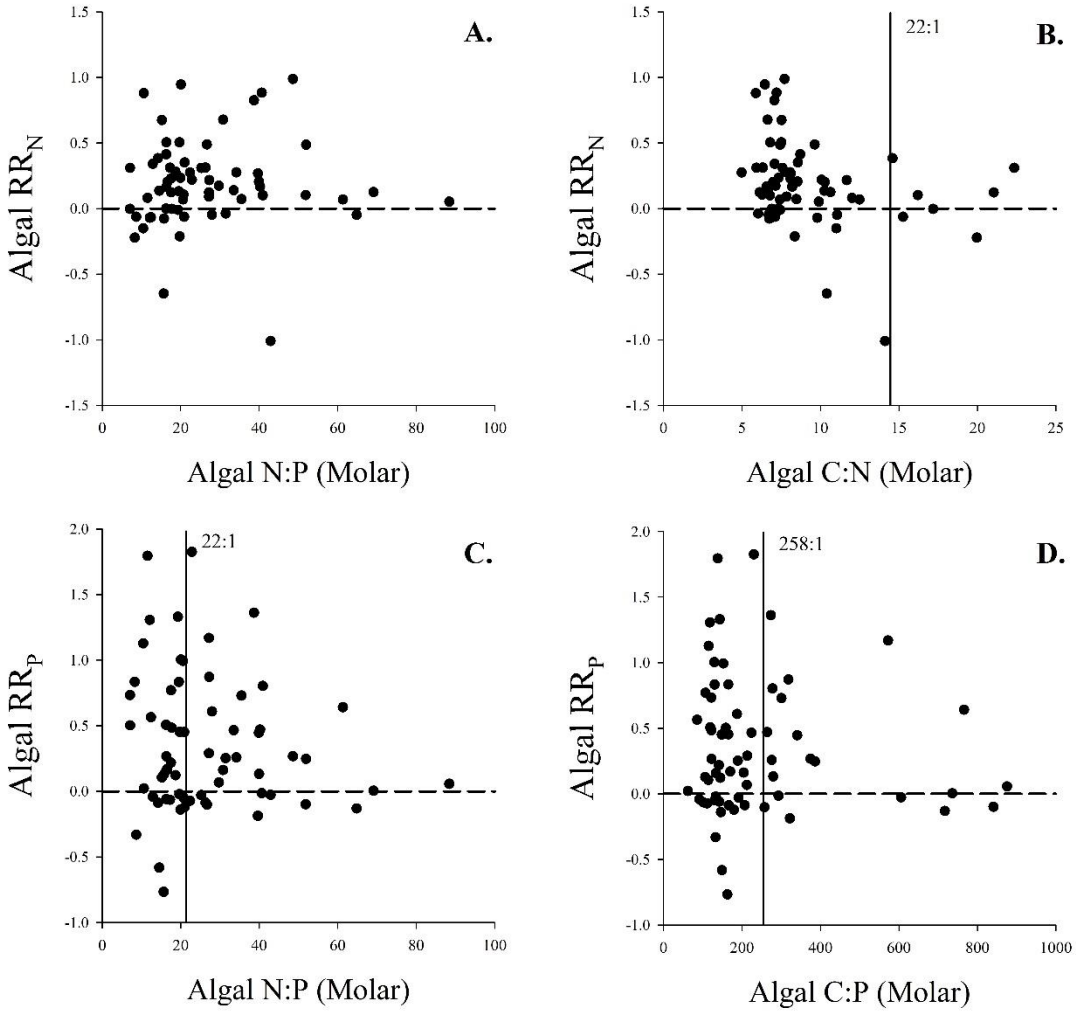

Figure 4. Nitrogen and Phosphorus response ratios (*RR*) as a function of molar stoichiometry within algae. (**A**) Algal nitrogen:phosphorus (N:P) versus algal phosphorus (N) *RRs*. and (**B**) with algal carbon: nitrogen ratios (C:N). (**C**) Algal phosphorus (P) *RRs* to algal N:P ratios and to (**D**) algal carbon to phorsphorus (C:P) ratios. Solid vertical black lines represent algal deficiency thresholds (Guildford and Hecky 2000). Data points indicate each individual lake sampling and date were used in these graphs from Ohio and central Texas, U.S.A reservoirs.

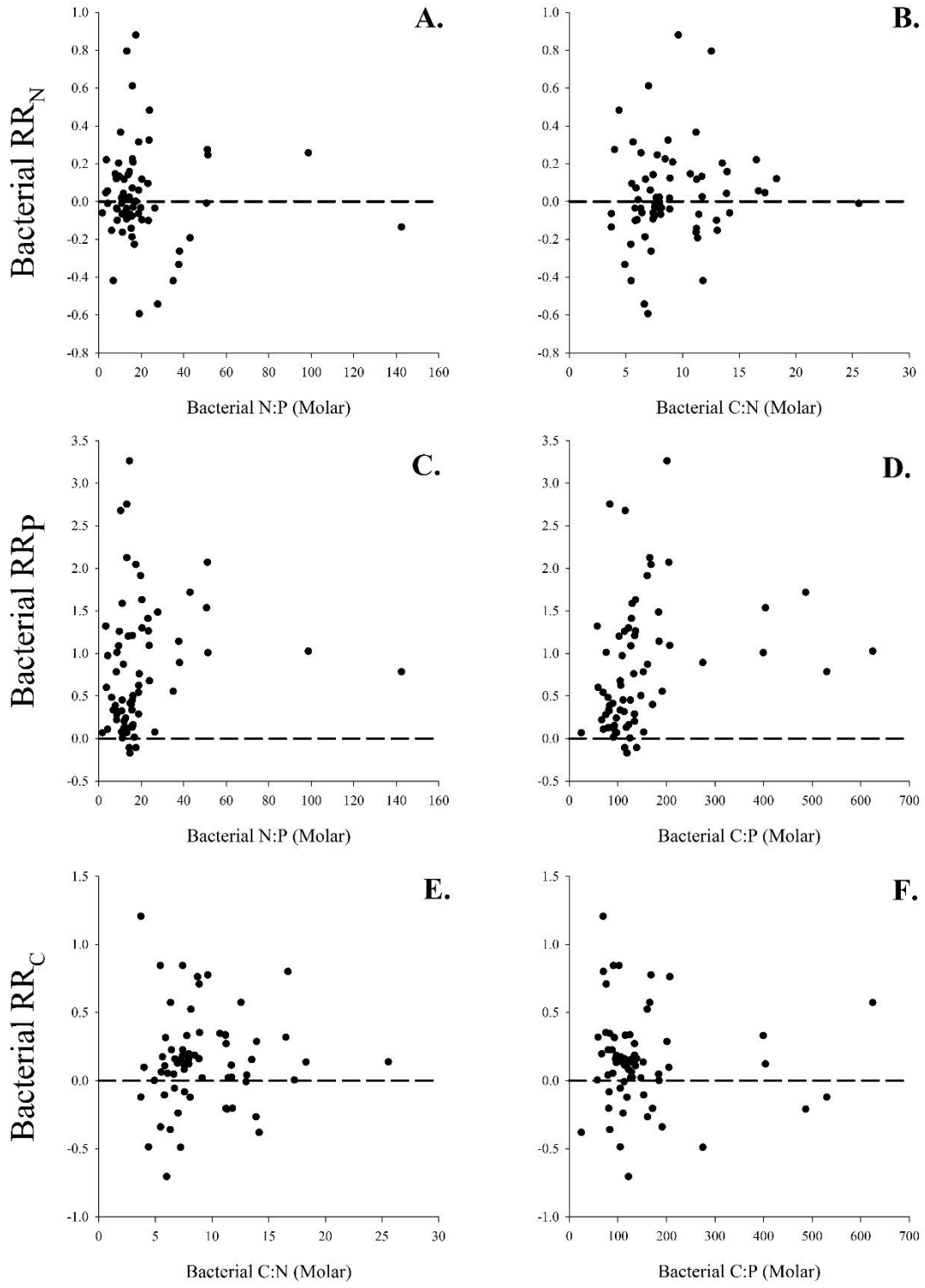

Figure 5. Three bacteria response ratios (*RR*) as a function of molar stoichiometry. (**A)** Bacterial carbon:nitrogen (C:N) and (**B**) carbon:phosphorus (C:P) in relation to bacterial carbon (C) *RRs*. (**C)** Bacterial nitrogen to phosphorus (N:P) and **(D**) C:P values when compared to bacterial phosphorus (P) RRs. And lastly, (**E**) bacterial C:N and (**F**) N:P ratios versus bacterial nitrogen (N) *RRs*. Data points signify each individual lake and date sampled in these graphs from Ohio and central Texas , U.S.A reservoirs.

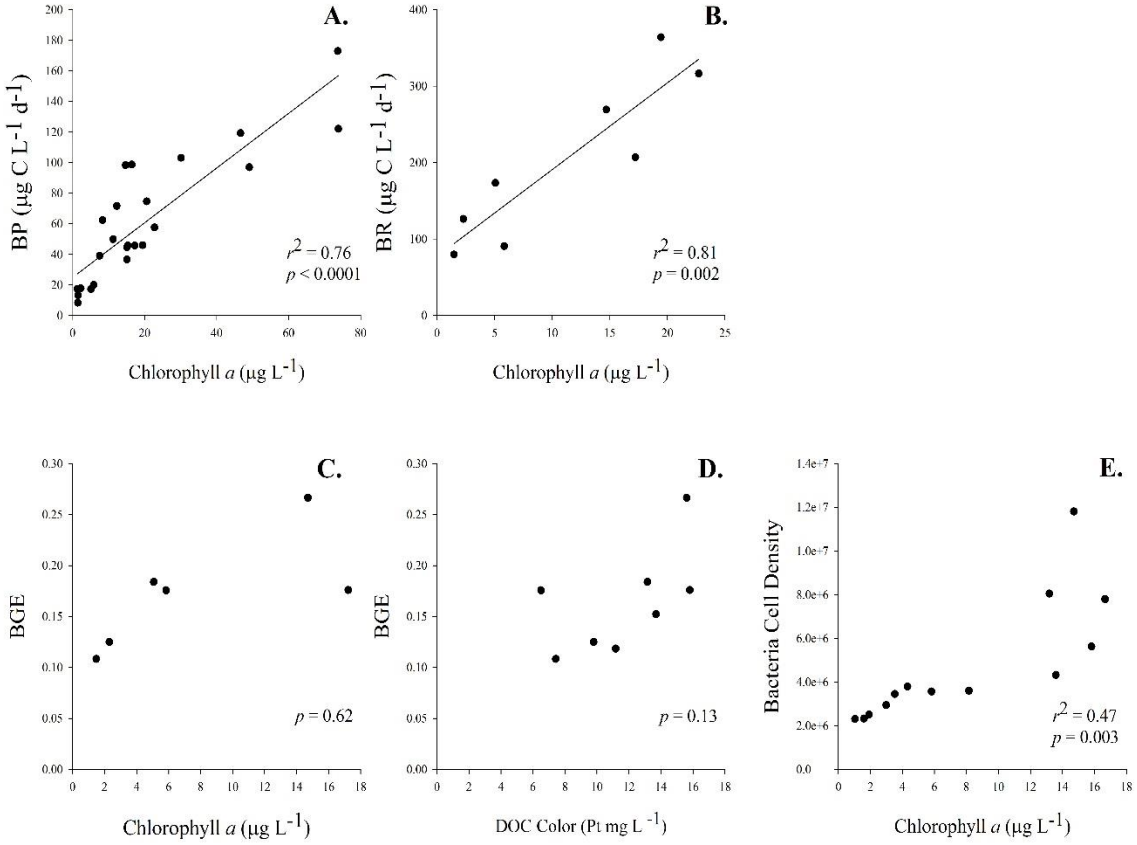

Figure 6. Chlorophyll *a* as a function of bacterial metabolism. (**A**) bacterial production (BPr) (**B**) bacterial respiration (BR), and (**C**) bacterial growth efficiency (BGE) as a proportion using the equation from del Giorgio and Cole (1998). (**D**) dissolved organic carbon color (DOC color) measured at 440nm as a relationship to BGE. Linear regression lines indicate significant relationships. Lastly, (**E**) Chlorophyll *a* as a function of bacteria cell density (number of bacteria mL^{-1}). Data points signify each individual lake and date sampled in these graphs from 2014 central Texas, U.S.A reservoirs.

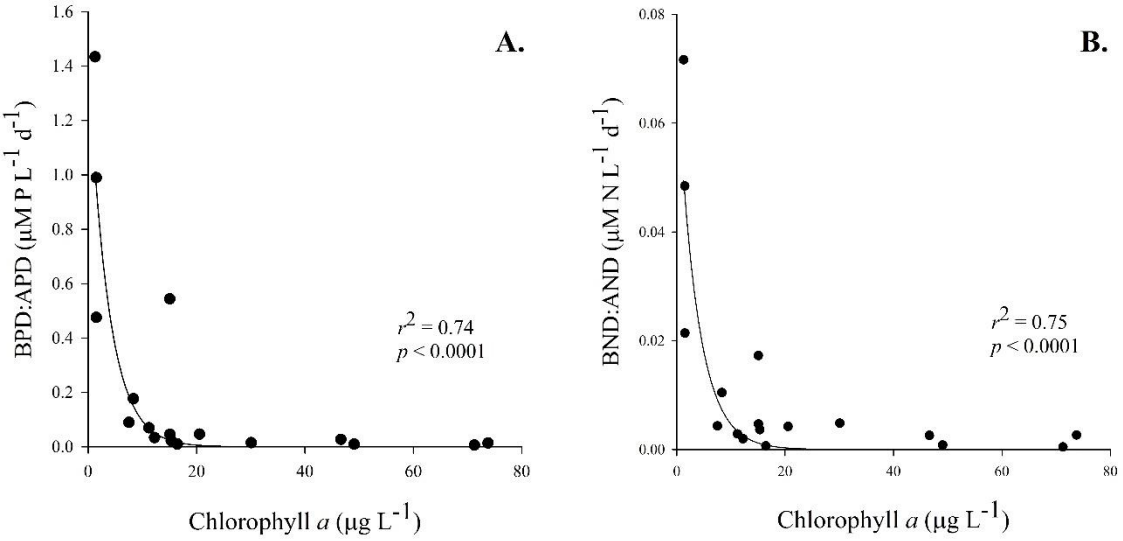

Figure 7. Competition and demand analysis across a trophic gradient. (**A**) Bacteria phosphorus demand (BPD) and algal phosphorus demand (APD) over a trophic gradient reducing to near minimum results and (**B**) bacterial nitrogen demand (BND) and algal nitrogen demand (AND) results in a similar relationship. Data for these analyses came from Caston et al. in which he had the PPr data available for Ohio and the 2006 central Texas reservoirs (2009).

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