Annual Progress Report for Project Entitled

Defining the balance between cyanobacterial N_2 fixation and denitrification in Falls of the Neuse Reservoir, NC

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The current nutrient response model for Falls Reservoir was developed by the NC Dept. of Environmental Quality-Division of Water Resources (DWR) and was used to substantiate a policy decision (Stage II of the Falls Reservoir Nutrient Management Strategy) of reducing N and P loads by 40 and 77%, respectively. The goal of the Nutrient Management Strategy is to reduce phytoplankton biomass to levels that meet the current NC water quality standard for chlorophyll *a* of 40 μg/L throughout Falls Reservoir. At the time this model was devised, there was substantial uncertainty in several model components including tributary inputs and sediment nutrient fluxes (Lin and Li 2011). The Upper Neuse River Basin Association (UNRBA) has contracted with Brown and Caldwell (B&C) to undergo a significant monitoring and modeling project aimed to reduce uncertainty in model predictions of phytoplankton biomass response to changing nutrient inputs. The monitoring component is largely completed. The modeling component began in spring 2019 with an initial focus on hydrology and physical processes that will be followed by water quality modeling (Matos, Alix, lead engineer for the B&C model, pers. comm.). As water quality modeling is being initiated, the UNRBA has requested third-party input into the design of the biogeochemical components of the water quality model sub-model.

The balance between N_2 fixation by cyanobacteria and N removal via denitrification is a critical driver of phytoplankton nutrient limitation in lakes and reservoirs (Scott and McCarthy 2010). Denitrification in shallow, highly productive lakes and reservoirs can remove significant quantities of N that in combination with efficient trapping of P can lead to strong N limitation (Grantz et al. 2014). N limitation has the potential to favor cyanobacteria groups capable of N_2 fixation. Examination of DWR's phytoplankton community composition data indicated that heterocystous cyanobacteria capable of N_2 fixation regularly comprise 25% or more of the phytoplankton biomass during the summer, but DWR's current water quality model does not contain a N_2 fixing cyanobacteria group. Omission of a N_2 fixing cyanobacteria group precludes the ability to simulate these N inputs to the reservoir and could create severe errors in estimation of phytoplankton biomass responses if N_2 fixation is an important process or could become quantitatively important if N inputs are reduced (Schindler et al. 2008).

Constraining N inputs by N_2 fixation will significantly enhance our understanding of phytoplankton nutrient responses in Falls Lake and will fill a significant data gap in the N mass balance for Falls Lake. Once this gap in the mass balance is filled, lake-wide N losses through denitrification can be calculated using a mass balance approach (Molot and Dillon 1993). Denitrification rates calculated by mass balance will complement direct measurements of

denitrification made on cores by Dr. Piehler's lab. Collectively, these efforts will provide significant data on water column and sediment N cycling to aid B&C in the formulation and parameterization of the updated nutrient response model.

METHODS

Sampling: Between late July 2019 and early July 2020, a series of five N_2 fixation assessment campaigns were conducted along a transect of 6 main-channel stations (Figure 1). Between May and September 2021, a series of four N_2 fixation assessments were conducted at ten stations located with major creek arms. During mid-channel sampling, integrated surface water from the surface to twice Secchi depth was collected from up two six stations that spanned the reservoir from just downstream of the I-85 bridge at Fish Dam Rd. (station A) to near the dam (station F) (Figure 1). During 2021, creek arms stations were sampled using surface grabs.

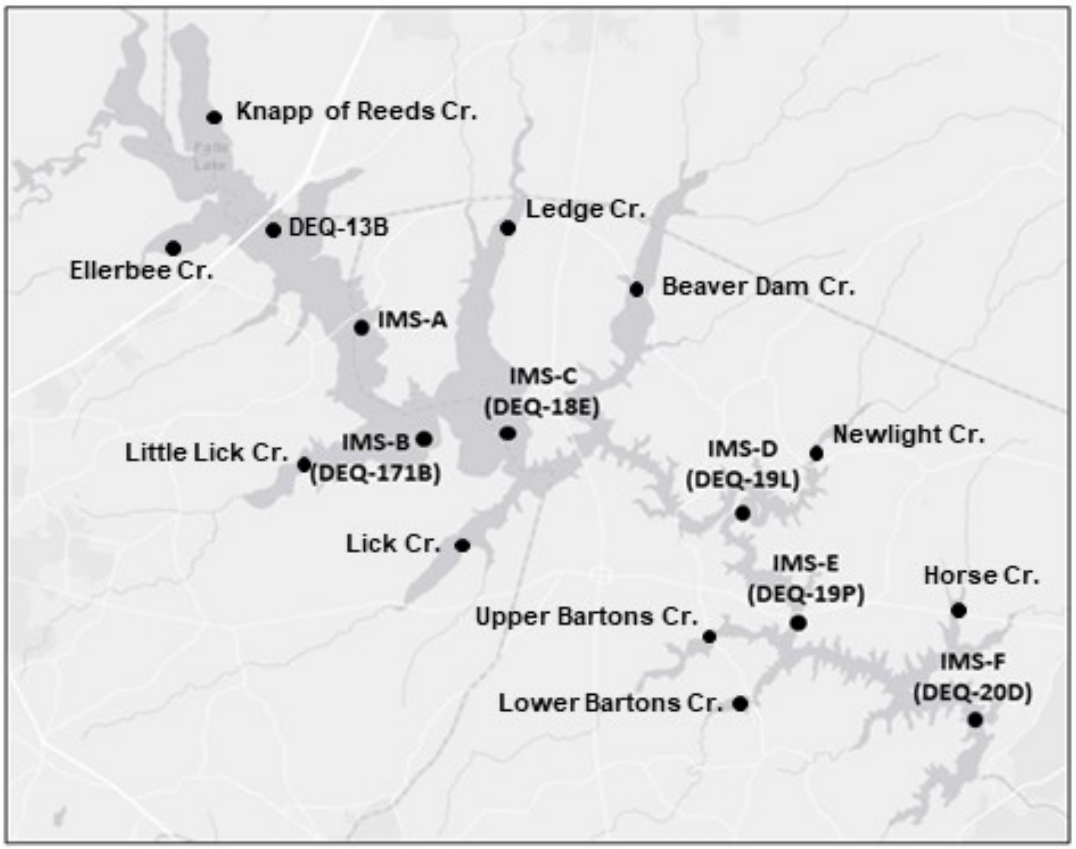

Figure 1. Map of main channel and creek sampling stations for measurements of N fixation rate. Five of the six main channel stations coincided with stations sampled monthly by NC Dept. of Environmental Quality (NCDEQ).

Measurement of water column N_2 fixation: During fall 2019 and summer 2020, N_2 fixation measurements made at mid-channel locations were made using acetylene reduction assays along the vertical light gradient according to Grantz et al. (2014) as follows. For each station, 50 mL aliquots of the photic zone composite sample were added to six, 70 mL clear-glass (borosilicate)

serum vials and a seventh vial that was made opaque by wrapping in black tape. Once each vial was stoppered using a rubber septum, four mL of head space was extracted from each vial and four mL of acetylene gas was injected. The six clear vials were suspended at depths from 0.25 m, 0.75 m, 1.25 m, 1.75 m, 2.25 m and 2.75 m. The seventh dark bottle from each station was incubated at 3 m depth. These in situ incubations maintained in situ temperature conditions, and spanned the light gradient from and bottles were suspended at several depths corresponding to ~50% to 0% of incident PAR. At each depth an additional bottle of deionized water was also amended with acetylene and identically incubated as a control to account for non-biological reduction of acetylene gas. At each depth the rate of non-biological acetylene reduction was subtracted off the measured acetylene reduction from all the stations. Incubations were conducted for three to four hours during midday. Rates of acetylene reduction were converted to rates of N_2 fixation assuming a 4:1 ratio of acetylene: N_2 reduction (Paerl et al. 2014).

For creek arm samples collected during 2021, N_2 fixation measurements were also made using the acetylene reduction technique but only at a single irradiance level (20% incident light). Surface waters from each station were collected in the morning. Aliquots of water from each sample were then incubated in triplicate clear serum bottles paired with triplicate dark bottles in a temperature-regulated water bath fitted with two layers of neutral density screening to reduce incident light to 20% of full-strength sunlight. Water temperature during the incubation was maintained at the in situ temperature observed at the last station sampled and deviated by less than 0.3 °C over the course of an approximate 4 hour incubation. As with the main channel measurements, acetylene reduction within triplicated deionized water controls was subtracted from all rate measurements to remove any non-biological acetylene reduction.

Measurement of nutrients, vertical structure of the water column, and phytoplankton biomass and community composition: At each sampling station, profiles of temperature, conductivity, dissolved oxygen, pH, and photosynthetically active radiation (PAR) were measured at the time of sampling using a YSI Exo multiparameter data sonde, and a LiCor PAR sensor. Aliquots of each photic zone composite sample were filtered through Whatman GF/F filters within a few hours of collection under gentle vacuum (< 7 in Hg) using a manual pump. The liquid filtrate was saved for dissolved nutrient analysis (ammonium, nitrate+nitrite, phosphate, total dissolved nitrogen, silicate) using a Lachat Quickchem 8000 autoanalyzer, while filters were retained for analysis of chlorophyll a by fluorometry, taxa specific accessory pigments by HPLC, and particulate carbon and nitrogen by elemental analysis (Peierls et al. 2012; Hall et al. 2013). Biomass of the four dominant phytoplankton classes in units of chlorophyll *a* were calculated from accessory photopigment concentrations using the matrix factorization program ChemTax (Mackey et al. 1996) following protocols from Paerl et al. (2014).

An aliquot of each sample was additionally preserved in Lugol's solution for species-level microscopic identification and enumeration of the phytoplankton community. Potentially N_2 fixing, heterocystous cyanobacteria within the order Nostocales were microscopically enumerated using inverted microscopy (Hall and Paerl 2011), and trichome biovolumes of each observed taxa were estimated based on measured lengths and widths measured using a calibrated, ocular Whipple grid and an assumed cylindrical morphology (Olenina et al. 2006).

From each sample, total biovolume of potential N_2 fixing cyanobacteria was computed as the sum of the biovolumes of all heterocystous cyanobacteria species present.

Examination of the relationships between directly measured rates of N_2 fixation by microscopically determined biomass of heterocystous cyanobacteria biomass allowed an estimate of the biomass-specific rate of N_2 fixation and comparing our observed biomass-specific N_2 fixation rates against literature values allowed us to assess how active the N_2 fixing cells of Falls Lake are compared to that observed in other systems, and whether observed variation in rates is likely due to changes in biomass or changes in biomass specific activity levels. The relationship developed between heterocystous cyanobacteria biomass and directly measured N_2 fixation was additionally used in conjunction with time series data of heterocystous cyanobacteria biomass collected by NCDEQ at four stations (Figure 1; NEU013B, NEU018E, NEU19P, NEU20D) to estimate an approximate monthly time series of N_2 fixation from 2011 to 2020.

Estimates of lake wide, annual N input due to N_2 fixation were calculated in two ways. The first method relied soley on the direct measurements of N_2 fixation. We first averaged across all depth-level incubations and then averaged all measurements across all stations including both the main channel and creek stations. This method is valid for producing a reasonable estimate of lake-wide N_2 fixation because both horizontal and vertical spatial gradients were weak. Average lake-wide measurements of N_2 fixation in units of mole N/L/h were multiplied by the total volume of the photic zone of Falls Lake (6.8×10^{10} L). The photic volume was calculated assuming an average photic zone depth of 1.5 m (UNRBA 2016) and using hypsographic data from UNRBA 2019 monitoring report (UNRBA 2019). To scale hourly measurements to annual estimates, we multiplied hourly rates by an assumed a 12 h photoperiod per day, and by 180 days per year when N_2 fixation is likely to occur (Grantz et al. 2014).

The second method of estimating annual, lake-wide N_2 fixation rates was conducted by scaling up and averaging the monthly time series of N_2 fixation estimated based on heterocystous cyanobacteria biomass at the four NCDEQ stations in the same way as the direct measurements. This time series of estimated N_2 fixation rates is based on the assumption that biomass-specific rates observed in our study were similar throughout the 2011-2020 period. While we have no data to validate this assumption, we also have no reason to suspect that it is not valid. Interannual variability of these biomass-based annual estimated N_2 fixation rates were compared against annual stream loads of N to investigate whether stream loads were related to N_2 fixation as would be expected if reduced N loads enhanced N limitation within the phytoplankton community.

Factors that controlled observed variability in rates of N_2 fixation were explored by comparing measured N_2 fixation rates against: 1) ambient nutrient concentrations, 2) cyanobacterial biomass determined by HPLC/ ChemTax, and 3) biomass of heterocystous cyanobacteria species.

Assessing nutrient limitation and effects of nutrient availability on N fixation: A set of nutrient addition bioassay experiments was conducted at three creek stations during spring and summer 2021 to determine the limiting nutrient in the creek arms and to determine the extent to which N_2 fixation is impacted by P availability. Additional (additional to samples used for N_2

fixation, nutrient and phytoplankton biomass/composition measurements) 60-liter water samples from Ledge, Knapp of Reeds, and Upper Barton Creeks were collected on 8 June and 19 July and 3 liters were dispensed into triplicated 4 liter Cubitainers. The triplicate Cubitainers were amended with the following treatments: a control with no added nutrients, nitrate addition, phosphate addition, and nitrate plus phosphate (see Paerl et al., 2014 for details). Growth in each treatment was assessed after 3 days and compared to initial measurements of phytoplankton biomass and community composition determined by HPLC pigments and microscopy. N_2 fixation rates were measured after 3 days incubation by acetylene reduction in light and dark bottles under two layers of neutral density screening in an outdoor water bath. Measuring N_2 fixation responses after 3 days primarily captures responses that are driven by changes in the physiology of the *in situ* cyanobacterial community versus responses that are increasingly likely to occur due to changes in the abundance of N_2 fixing taxa. To determine the degree to which P availability can regulate changes in N fixation, control and phosphate addition treatments were continued through a full week (7- 8 days), and biomass, community composition, and N_2 fixation were measured again. This information is useful for determining the potential for P inputs to stimulate N_2 fixation and can provided a useful upper constraint for modeled N_2 fixation rates (Del Guidice and Obenour 2021).

Characterizing the N mass balance: Annual tributary loads of total N and total P for tributaries to Falls Lake and atmospheric deposition of N over the period 2006 to 2019 were taken from NCDEQ's 2021 Status Report of the Falls Lake Nutrient Strategy (NCDEQ 2021). The tributary load estimates were based on LOADEST models of load from the major gaged tributaries, and estimates of atmospheric deposition to the lake surface were based on interpolations of total inorganic nitrogen deposition within the US EPA's Clean Air Status and Trends (CASTNET) monitoring network (NCDEQ 2021). Annual fluxes of total N and total P out of Falls Lake were calculated using the weighted regressions on time, discharge, and season (WRTDS) model (Hirsch et al. 2010) on USGS gaged discharge (USGS gage 02087183) and monthly concentration data collected by NC DEQ's Ambient Monitoring System (station J1890000). Annual N inputs were calculated as the sum of tributary loads, atmospheric deposition, and N_2 fixation. Net retention of N (TN_{ret}, units kg N/y) and P (TP_{ret}, units kg P/y) was determined as the difference between annual inputs and outputs through river flux. Under an assumption that net retention of P is due solely to sedimentation, the whole lake denitrification rate (DNF) can be estimated based on the ratio of N:P retention (N/P_{ret}) and the average N:P mass ratio of the lake's surface sediments (N/P_{sed}) (equation 1; Molot and Dillon 1993).

Equation 1. $$DNF = TN_{ret}(N/P_{ret} - N/P_{sed})/(N/P_{ret})$$

The lake-wide sediment N:P mass ratio was calculated as 3.67 (+/-1.25 S.D.) based on the average N:P ratio of the upper, 0-3 cm, sediment layer within 30 cores collected by Dr. Alperin. These 30 cores spanned the entire length of the reservoir and included both shallow and deeper channel locations. Although the TN:TP ratio of the surface sediments varied from 1.0 to 5.7, there were no consistent patterns of the ratio in relation to distance downstream or depth of overlying water (Alperin 2018).

RESULTS

Rates of N_2 fixation generally ranged from 0 to 5 nmol N/L/h (Fig. 2) with an average of 1.3 and standard deviation of 1.4 nmol N/L/h. The highest observed rate was 10.1 nmol N/L/h in a sample collected in May 2020 from station A and incubated just below the surface at 0.25 m depth. There was a general but weak trend toward higher rates of N_2 fixation in samples incubated closer to the surface. The relationship of N_2 fixation to incubation depth (i.e. light availability) was particularly evident in samples that had higher rates of N_2 fixation such as station E collected on 7/22/2019 and 10/14/2019 and samples collected from stations C through F on 7/7/2020. A Spearman rank correlation between incubation depth and N_2 fixation revealed a

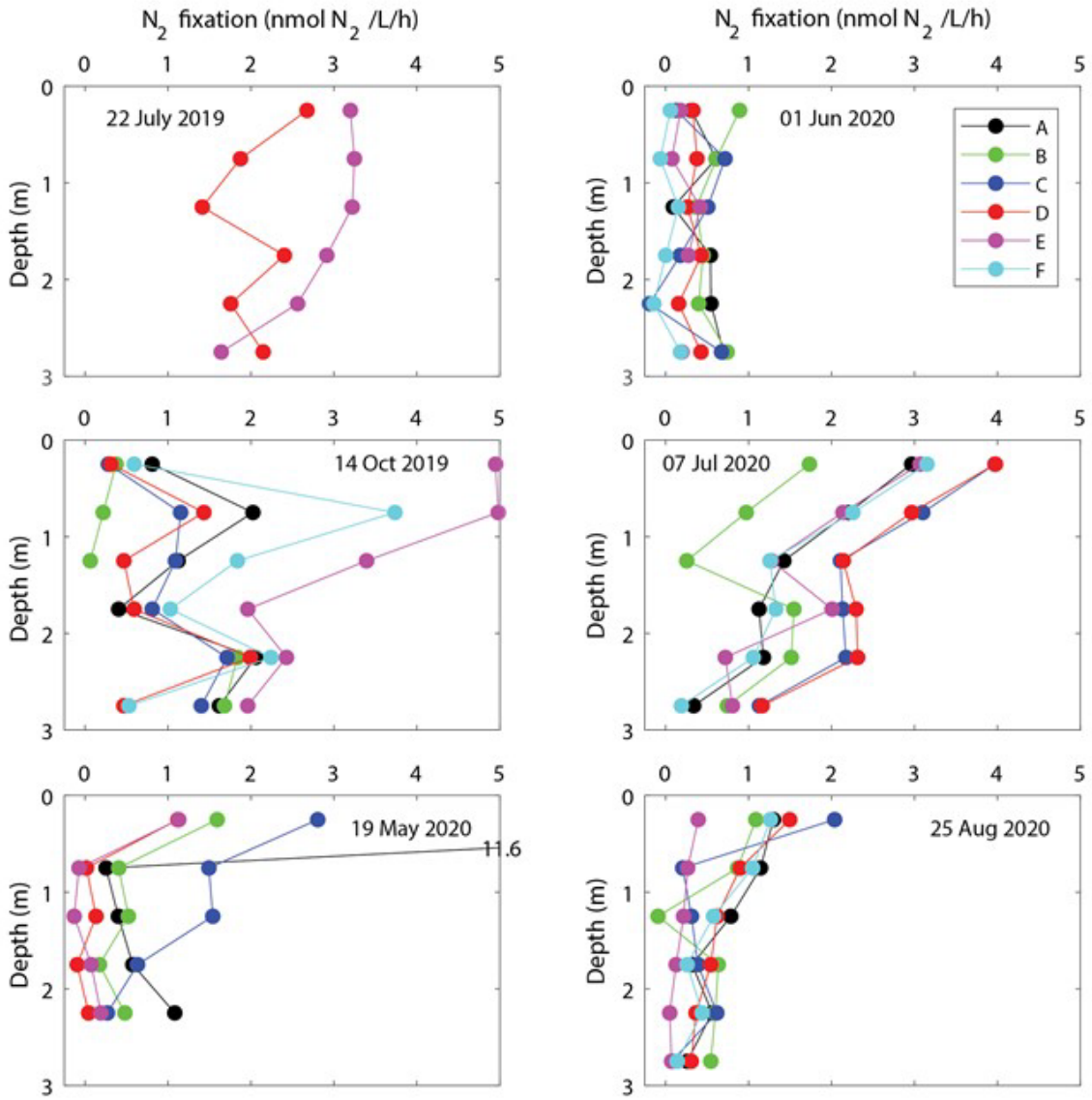

Figure 2. Depth profiles of N fixation measured at 6 mid-channel stations A-F in Falls Lake during project year 2019-2020.

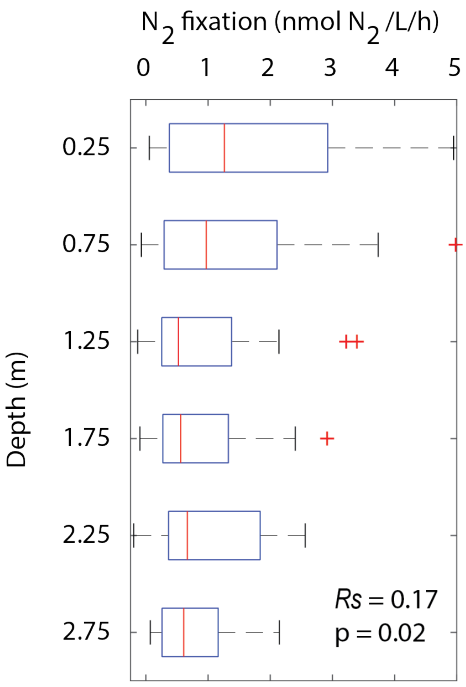

Figure 3. Boxplots of N2 fixation measured on main channel samples incubated at six depths. Red lines indicate median values. Boxes represent the interquartile range. Whiskers extend a distance 1.5 times the interquartile range from the median and red + symbols identify values outside the whiskers.

weak but statistically significant increase of N_2 fixation at shallower, well-lit depths (Figure 3; *Rs*= 0.17, $p = 0.02$). This indicates that light availability is likely a constraint on N_2 fixation in Falls Lake.

Along the axis of the lake, the median values of main channel N_2 fixation showed no clear pattern and were all very close to 1 nmol N/L/h (Figure 4). To date, four assessments of creek arm N_2 fixation have been conducted at 10 sites from June through august 2021 and results from three assessments are presented in Figure 5. Rates of N_2 fixation ranged from -2 to 12 nmol N/L/h. The negative values occurred on 8 June and 19 July when the acetylene reduction of the deionized water control was higher than normal. With an average of 3.2 (median of 2.8 nmol N/L/h) the creek arm stations generally had higher rates of N_2 fixation than was measured at mid-channel locations. Among the creeks, it appears that creeks on the eastern side of the lake including Horse, Newlight, Beaver Dam and Ledge Creeks tend to have lower rates of N fixation than creeks on the western side of the reservoir. This finding may relate to differences in nutrient loading from the more highly developed watersheds on the western side of the lake, and will be explored further in the coming year's work.

For the main channel stations, relationships between maximum N_2 fixation across depths and bioavailable, inorganic N and P nutrient forms (nitrate, ammonium, and phosphate), and cyanobacteria biomass estimated by HPLC accessory pigment concentrations have been explored using Spearman's rank correlations to improve understanding of the controls on N_2 fixation in Falls Lake. Future analyses will incorporate data collected during 2021 from the creek arm samples. Maximum N_2 fixation rate within each profile (i.e. the N_2 fixation rate at an optimal irradiance) was used as a dependent variable rather than a depth-averaged rate to help separate the potential influence of light limitation of

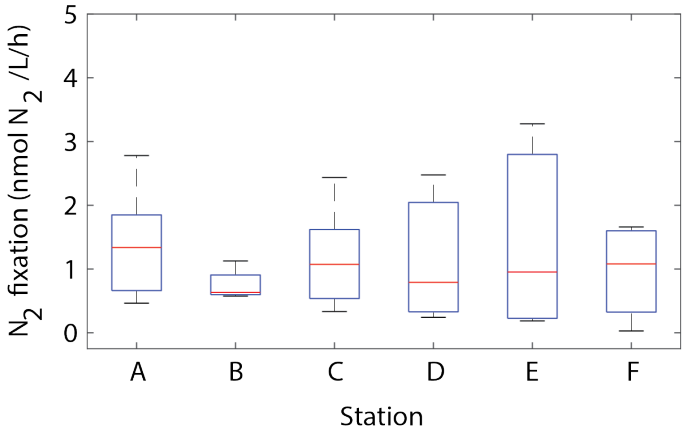

Figure 4. Boxplots of N2 fixation measured at six main channel. Red lines indicate median values. Boxes represent the interquartile range. Whiskers extend a distance 1.5 times the interquartile range from the median and red + symbols identify values outside the whiskers.

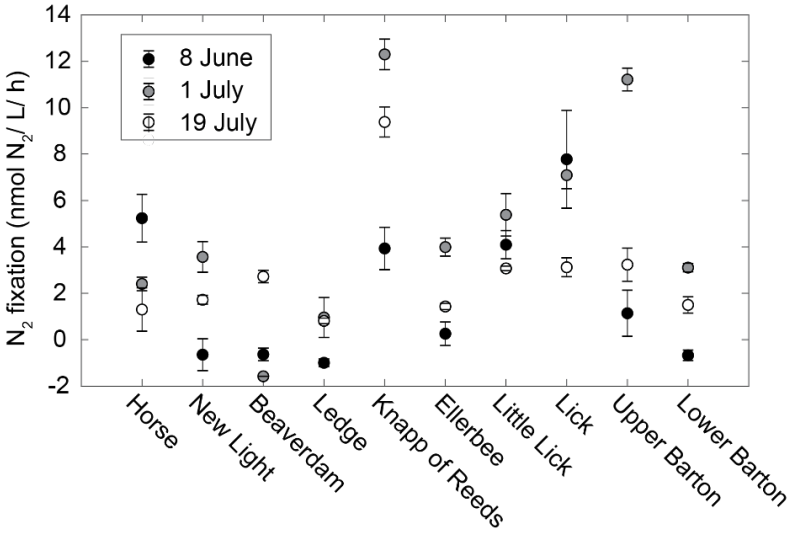

Figure 5. N2 fixation rates measured in Falls Lake creek arms during spring and summer 2021. Circles are means of tripicate values and error bars represent the standard deviation.

N_2 fixation from other factors. The relationships between maximum N_2 fixation within each profile exhibited no significant relationships to nitrate (Fig. 6). However, for most samples nitrate was below detection, and a value of half the detection limit (0.35 mg/L) was assumed. A statistically-significant, negative relationship was were observed between ammonium and N_2 fixation. A negative relationship of N_2 fixation with ammonium is expected because elevated ammonium is known to inhibit

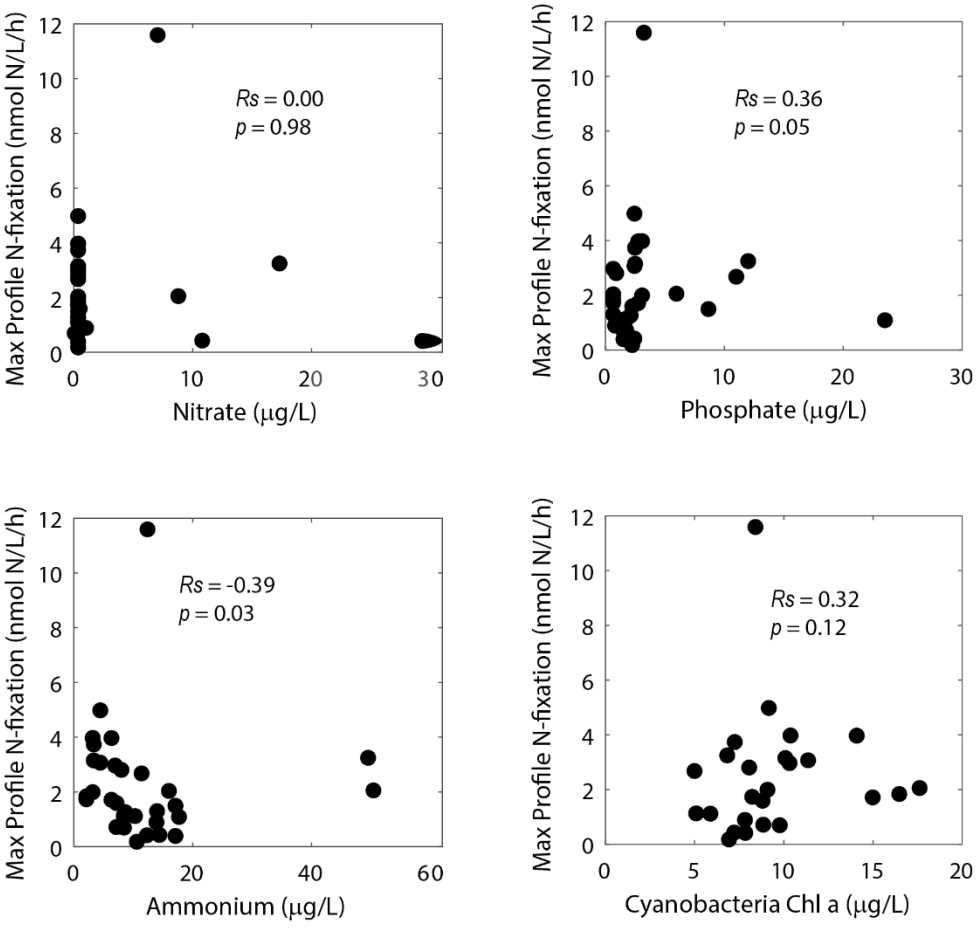

Figure. 6. Scatter plots and rank correlations of the maximum N_2 fixation rate measured along each depth profile of the incubations used to measure main N_2 fixation versus nutrients and cyanobacterial biomass measured by HPLC/Chemtax.

synthesis of the nitrogenase enzyme complex required for N_2 fixation (Agawin et al. 2007). Phosphate exhibited a statistically-significant positive relationship to N_2 fixation, and these results are consistent with the current paradigm that N_2 fixation is promoted under conditions of low N and high P availability.

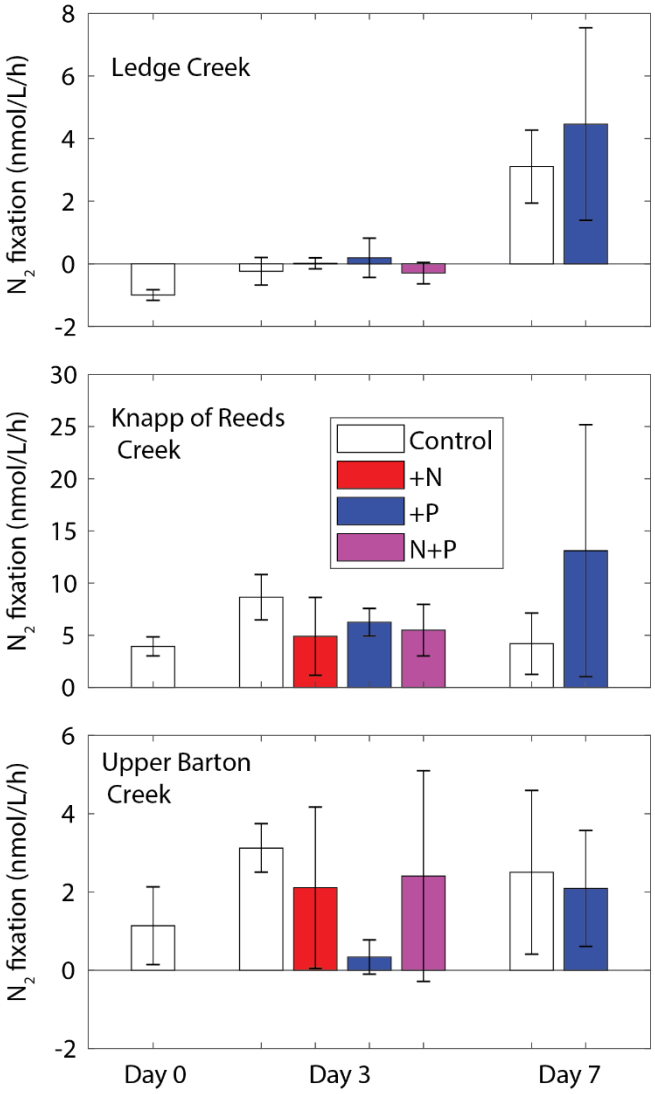

Figure 7. Response of N_2 fixation to N and P ammendments in an experiment conducted from Falls Lake creek arm waters in June 2021.

Initial results from the nutrient addition experiment conducted on 8 June generally corroborate the positive effect of P availability on stimulating N_2 fixation (Figure 7). For both Ledge and Knapp of Reeds Creek, highest rates of N_2 fixation were observed after a week long incubation in the phosphate addition treatment though with a high degree of within-treatment variability. Strangely, the P addition appeared to depress N_2 fixation on day 3 of the experiment in Upper Barton Creek. Samples for phytoplankton biomass and community composition by HPLC pigments, particulate N and C, and phytoplankton microscopy were collected and are currently being analyzed as are N_2 fixation data from the bioassay experiment conducted on 19 July 2021. Closely examining changes in phytoplankton community composition during the experiments will help elucidate the linkages between N_2 fixation and community compositional responses to nutrient manipulations.

The relationship between cyanobacterial biomass determined by HPLC pigment analyses and N_2 fixation was not statistically significant (Figure 6). This was not terribly surprising because many of the cyanobacteria known to dominate Falls Lake cyanobacteria assemblages are non-N_2 fixing members of the *Oscillatoriales* and *Chroococcales* (Touchette et al.2007). The biomass of potentially N_2 fixing, heterocystous taxa in the order *Nostocales* was quantified microscopically and exhibited a much stronger (R_S = 0.69) and statistically significant relationship ($p < 0.0001$) with rates of N_2 fixation (Figure 8).

Biovolume of potential N_2 fixing cyanobacteria ranged from 0 to ~2.8 10^9 μm^3/ L (Figure 8). A zero-intercept linear regression of Nostocalean cyanobacteria biovolume versus rate of N_2

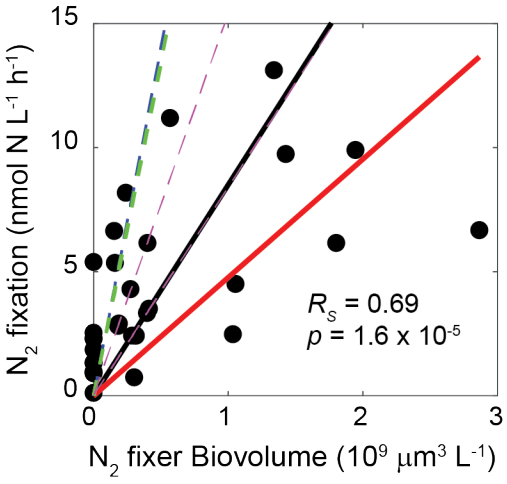

Figure 8. Measured rates of N_2 fixation versus biomass of heterocystous cyanobacteria at main channel sites on Falls Lake. (filled black circles). Solid red line represents a zero-intercept, least square regression. Solid black line represents the median ratio of N_2 fixation: N_2 fixer biovolume after excluding samples with zero N_2 fixer biovolume. Dashed blue, green, and the two magenta lines represent ratios respectively observed by Klawonn et al. (2016) from the Baltic Sea, Willis et al. (2016) from a culture of *Cylindrospermopsis raciborskii*, and Moisander et al. (2012) from *C. raciborskii* dominated assemblages from Lake George and the St Johns River, Florida.

fixation was statistically significant (p = 0.001) but only explained 31 % of the variability in the rate of N_2 fixation. The relationship between heterocystous cyanobacteria biomass and N_2 fixation was compared with literature values. A zero-intercept, least square fit (red line in Figure 4) produced a relationship with a lower N_2 fixation rate: biomass ratio than observed from the literature. The least square regression line appeared highly influenced by the highest biomass data point which only had modest N_2 fixation. The median ratio of N_2 fixation to N_2 fixer biovolume was more similar to literature values, especially the *Cylindrospermopsis raciborskii*- dominated natural assemblages (magenta lines in Figure 4) measured by Moisander et al. (2012). The N_2 fixer assemblage of Falls Lake was also dominated by *C. raciborskii* (Figure 5) and moderate biomass-specific N_2 fixation rates are likely typical of this species (Moisander et al. 2012; Willis et al. 2016). Based on agreement with previous literature values and the high influence of a few data points on the least square regression, all estimates of N_2 fixation rates based on heterocystous cyanobacteria biomass were made using the median ratio of observed N_2 fixation vs biomass, 8.5×10^{-9} nmol N/µm^3 N-fixer biovolume/h.

Estimates of N_2 fixation rates based on heterocystous cyanobacteria biomass measured by NCDEQ varied seasonally from about 0.1 nmol N/L/h during winter to above 10 nmol N/L/h during summer (Figure 9). One of the dangers of inferring rates from biomass of a large group species that likely have different levels of N_2 fixation activity (Moisander et al. 2012) is that community composition may vary between when rate measurements are made

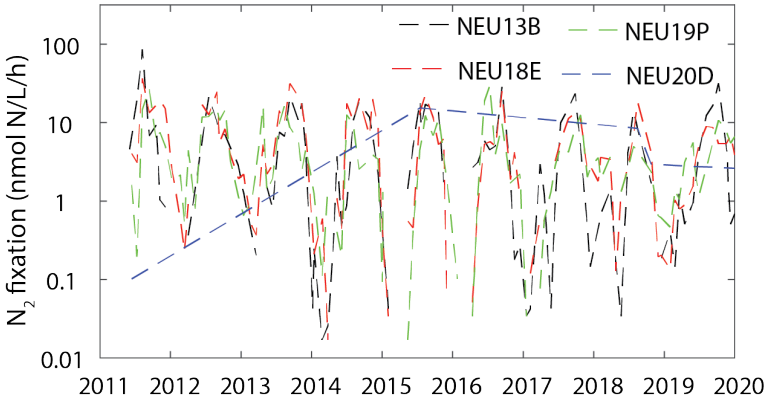

Figure 9. Time series of estimated N2 fixation at four locations in Falls Lake based on the product of heterocystous cyanobacterial biomass measured by NCDEQ and the median biomass-specific N2 fixation rate measured in this study from main-channel sites.

and the time period over which the biomass-specific rates are applied to estimate the time series of N_2 fixation. The heterocystous cyanobacteria community of the main channel samples collected by this study during 2019 and 2020 is very similar to that observed by NC DEQ over the period 2011 to 2018. *Cylindrospermopsis raciborskii* is dominant followed by *Dolichospermum sp.*, *Aphanizomenon sp.* and then a small fraction composed by other members of the order Nostocales. This lessens the probability that significant changes in community composition of the heterocystous cyanobacteria community could lead to significant error or bias in the N_2 fixation estimates derived biomass of heterocystous cyanobacteria.

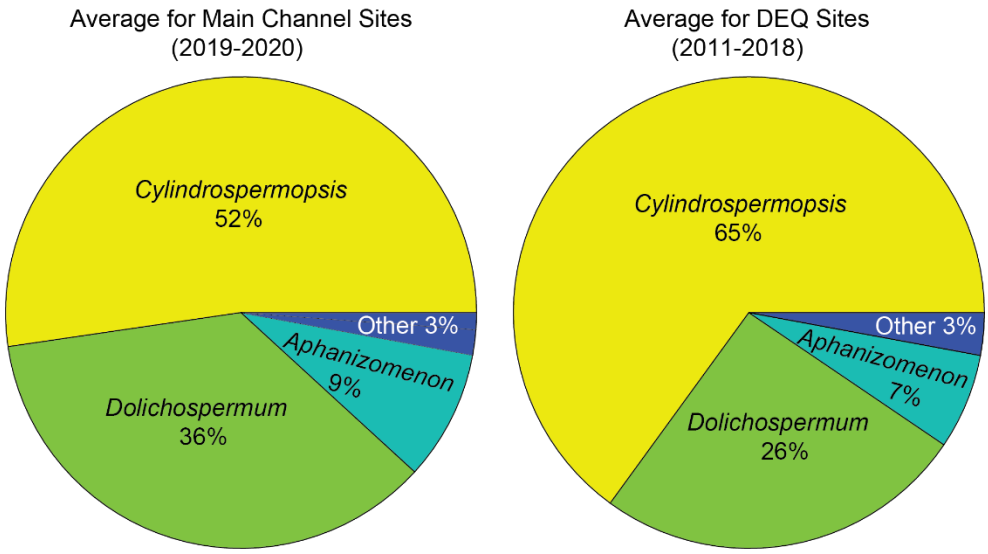

Figure 10. Average composition of heterocystous, potential N_2 fixing cyanobacteria taxa for the main channel sites in this study and from NC DEQ's stations NEU013B, NEU018E, NEU019P, and NEU020D.

N mass balance: Based on the average values of direct measurements of N_2 fixation from the main channel and creek arm sites and the assumptions used to scale our bottle incubation measurements up to lake-wide annual rates (see methods), the annual N input due to N_2 fixation is ~6600 kg N/ y (Table 1). Estimates of lake-wide annual N_2 fixation rates estimated based on heterocystous cyanobacteria biomass ranged from 14,000 to 34,000 kg N/y and averaged 23,000 kg N/y, approximately four fold higher than the annual estimate based on scaling up direct measurements. Thus, depending on which method is used, N_2 fixation supplied an average of ~1% of tributary N loads if the direct measurements are used (Table 1) versus an average of ~5% if the estimates based on cyanobacteria biomass are used (Table 2).

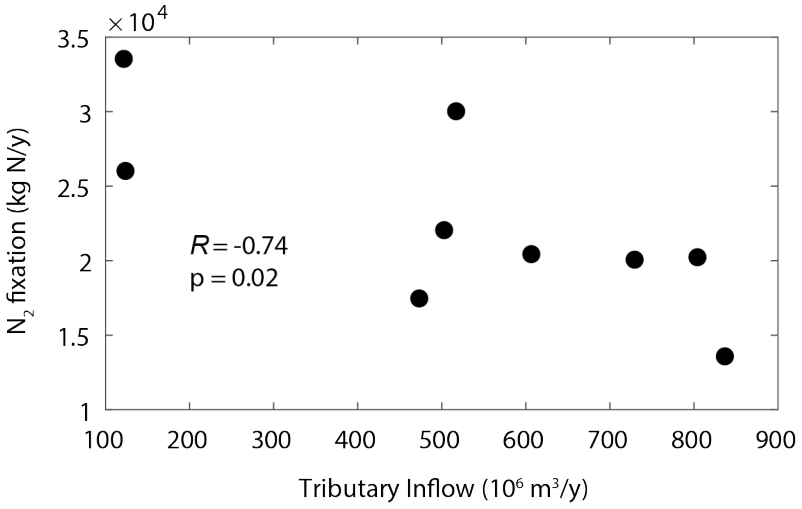

Figure 11. Annual N2 fixation rate versus annual tributary inflow to Falls Lake. Annual N2 fixation rate was estimated based on the product of heterocystous cyanobacterial biomass measured by NCDEQ and the median biomass-specific N2 fixation rate measured in this study from main-channel sites.

In dry years like 2011 and 2012 that had low tributary loads, estimates based on cyanobacteria biomass indicated that N_2 fixation may equal up to 16% of the tributary N load, roughly the same magnitude as atmospheric deposition (Table 2). The increased relative importance of N_2 fixation during dry years was not only caused by decreases in tributary loads. There also was a general trend of increasing magnitude of estimated N_2 fixation under low flow conditions (Figure 11) which might indicate compensation of N deficits when tributary N loads are low.

The annual estimates of N_2 fixation derived using both methods was added to annual estimates of tributary N loads and atmospheric deposition to develop a mass balance for N for the years 2006 to 2019 (Table 1). First, we describe the nutrient budget obtained using the scaled-up direct measurements to produce a constant annual average N_2 fixation rate, and we subsequently contrast that nutrient budget with the budget derived from annually-variable N_2 fixation estimates based on heterocystous cyanobacterial biomass.

Using scaled-up direct measurements of N_2 fixation, total N inputs to Falls Lake including tributary inputs, atmospheric deposition, and N_2 fixation ranged from 2.7×10^5 in 2011 which was a very dry year to 8.9×10^5 kg N/y during 2018, a very wet year (Table 1). The percent of total N inputs removed by sedimentation and denitrification in Falls Lake varied from -9% in 2016 to 69% in 2011 and 2012, and averaged 35% (Table 1). As is often the case, the percentage of tributary loads of TP that were retained by Falls Lake was considerably higher than for TN, ranging from 41 to 88%, and averaging 65% (Table 1). When the scaled-up direct N_2 fixation The mass ratio of TN to TP inputs retained in Falls Lake varied from 0.25 in 2016 to 8.4 in 2015 and averaged 4.4 (data not shown). On average the ratio of TN to TP retention was higher than the mass ratio of TN:TP in the surficial sediments which is indicative of N losses by denitrification (Molot and Dillon 1993). Lake-wide denitrification calculated via equation 1 ranged from -1.4×10^5 to 1.4×10^5 kg N/y and averaged 3.8×10^4 kg N/y (Table 1). In comparison to the total tributary loads of N, denitrification represented between -24 and 51%, and averaged a modest 9% of the total annual tributary loads of N (Table 1).

For the nutrient budget that was derived using N_2 fixation estimates derived from heterocystous cyanobacterial biomass, the approximately four-fold higher of estimate N inputs and negative relationship with tributary nutrient loading resulted in small increases in estimated rates of

denitrification compared to the budget based on scaled-up direct measurements of N_2 fixation, average of 4.1×10^4 versus 3.8×10^4 kg N/y (Table 2). However, higher estimates of N_2 fixation increased average denitrification as a percent of tributary loadings from 9 (Table 1) to 14% (Table 2).

The finding that there was essentially no N retention during 2016 may be an artifact created by underestimation of tributary N inputs or overestimation of river fluxes of N out of Falls Lake. Outflowing TN flux estimated here using the WRTDS method was 5% lower than the outflowing TN flux estimated by NC DEQ using the LOADEST model (NCDEQ 2019). So, it appears more likely that the unusually low retention of N during 2016 is caused by an underestimation of N inputs. It is also possible that storms in late December 2015 led to large nutrient loads that were stored in the lake and slowly released during the early part of 2016. These potential explanations for the unusual N balance for 2016 will continue to be explored, and may provide valuable insights into the dynamics of N fluxes into and out of Falls Lake.

Table 1. Mass balance of total N and total P and calculations of lake-wide annual denitrification for the period 2006-2019 using a constant annual N2 fixation rate derived from direct measurements made by this study.

Year	Trib. N load (kg N/y)	Atm. dep. (kg N/y)	N_2 fix. (kg N/y)	Total TN flux in (kg N/y)	River TN flux out (kg N/y)	TN retain (%)	Trib. P load (kg N/y)	River TP flux out (kg P/y)	TP retain (%)	TN:TP retain	Denit. (kg N/y)	Denit. (% Trib. Load)
2006	4.3×10^5	6.3×10^4	6.6×10^3	5.0×10^5	2.3×10^5	55	7.9×10^4	1.3×10^4	84	4.2	3.4×10^4	7
2007	3.1×10^5	2.7×10^4	6.6×10^3	3.5×10^5	2.6×10^5	26	4.1×10^4	1.6×10^4	61	3.6	-1.5×10^3	0
2008	5.2×10^5	4.2×10^4	6.6×10^3	5.7×10^5	2.0×10^5	64	8.6×10^4	1.1×10^4	88	4.9	8.9×10^4	16
2009	6.7×10^5	4.6×10^4	6.6×10^3	7.2×10^5	4.6×10^5	36	7.7×10^4	2.6×10^4	67	5.1	7.2×10^4	10
2010	4.8×10^5	4.6×10^4	6.6×10^3	5.3×10^5	3.6×10^5	32	5.3×10^4	2.1×10^4	60	5.3	5.2×10^4	10
2011	2.0×10^5	4.6×10^4	6.6×10^3	2.6×10^5	8.3×10^4	68	1.6×10^4	3.9×10^3	75	15.0	1.3×10^5	51
2012	2.1×10^5	4.6×10^4	6.6×10^3	2.6×10^5	8.6×10^4	68	1.7×10^4	3.8×10^3	77	13.9	1.3×10^5	50
2013	4.7×10^5	2.8×10^4	6.6×10^3	5.1×10^5	3.8×10^5	25	4.5×10^4	2.0×10^4	57	5.0	3.3×10^4	7
2014	5.1×10^5	2.6×10^4	6.6×10^3	5.5×10^5	4.7×10^5	14	5.0×10^4	2.6×10^4	48	3.1	-1.3×10^4	-2
2015	5.3×10^5	6.1×10^4	6.6×10^3	6.0×10^5	4.0×10^5	34	5.5×10^4	2.1×10^4	62	6.0	7.8×10^4	13
2016	5.2×10^5	5.9×10^4	6.6×10^3	5.8×10^5	6.4×10^5	-9	5.9×10^4	3.5×10^4	41	-2.2	-1.4×10^4	-24
2017	4.8×10^5	5.4×10^4	6.6×10^3	5.4×10^5	3.4×10^5	37	6.9×10^4	1.9×10^4	73	4.0	1.7×10^4	3
2018	8.2×10^5	5.4×10^4	6.6×10^3	8.8×10^5	6.4×10^5	27	1.1×10^5	3.3×10^4	70	3.1	$-.4.5\times10^4$	-5
2019	6.0×10^5	5.7×10^4	6.6×10^3	6.6×10^5	6.0×10^5	10	6.5×10^4	3.3×10^4	50	1.9	-5.6×10^4	-9
Avg	**4.8×10^5**	**4.7×10^4**	**6.6×10^3**	**5.4×10^5**	**3.7×10^5**	**35**	**5.9×10^4**	**2.0×10^4**	**65**	**5.2**	**2.7×10^4**	**9**

Table 2. Mass balance of total N and total P and calculations of lake-wide annual denitrification for the period 2006-2019 using annual N2 fixation rates estimated based on biomass of potentially N-fixing cyanobacteria quantified by NCDEQ and biomass-specific rates of N2 fixation measured in this study.

Year	Trib. N load (kg N/y)	Atm. dep. (kg N/y)	N_2 fix. (kg N/y)	Total TN flux in (kg N/y)	River TN flux out (kg N/y)	TN retain (%)	Trib. P load (kg N/y)	River TP flux out (kg P/y)	TP retain (%)	TN:TP retain	Denit. (kg N/y)	Denit. (% Trib. Load)
2011	2.0×10^5	4.6×10^4	3.4×10^4	2.8×10^5	8.3×10^4	71	1.6×10^4	3.9×10^3	75	15.0	1.6×10^5	77
2012	2.1×10^5	4.6×10^4	2.6×10^4	2.8×10^5	8.6×10^4	70	1.7×10^4	3.8×10^3	77	13.9	1.5×10^5	71
2013	4.7×10^5	2.8×10^4	3.0×10^4	5.3×10^5	3.8×10^5	28	4.5×10^4	2.0×10^4	57	5.0	5.7×10^4	12
2014	5.1×10^5	2.6×10^4	2.0×10^4	5.6×10^5	4.7×10^5	16	5.0×10^4	2.6×10^4	48	3.1	1.2×10^3	0
2015	5.3×10^5	6.1×10^4	2.2×10^4	6.2×10^5	4.0×10^5	36	5.5×10^4	2.1×10^4	62	6.0	9.4×10^4	18
2016	5.2×10^5	5.9×10^4	2.0×10^4	6.0×10^5	6.4×10^5	-7	5.9×10^4	3.5×10^4	41	-2.2	-1.3×10^5	-25
2017	4.8×10^5	5.4×10^4	1.8×10^4	5.5×10^5	3.4×10^5	38	6.9×10^4	1.9×10^4	73	4.0	2.8×10^4	6
2018	8.2×10^5	5.4×10^4	1.4×10^4	8.9×10^5	6.4×10^5	28	1.1×10^5	3.3×10^4	70	3.1	$-.3.8\times10^4$	-5
2019	6.0×10^5	5.7×10^4	2.0×10^4	6.7×10^5	6.0×10^5	11	6.5×10^4	3.3×10^4	50	1.9	-4.3×10^4	-7
Avg	**4.8×10^5**	**4.8×10^4**	**2.3×10^4**	**5.5×10^5**	**4.0×10^5**	**32**	**5.4×10^4**	**2.1×10^4**	**62**	**6.3**	**3.1×10^4**	**12**

Comparison of mass balance estimates vs. direct measurements of denitrification

To date, Dr. Piehler's laboratory has conducted four campaigns to directly measure rates of sediment denitrification in Falls Lake. These campaigns were conducted during October 2019, May 2020, August 2020, and July 2021 in conjunction with surface water sampling from the six main channel and ten creek arm sites to measure N_2 fixation. Average values of denitrification for the first three campaigns have been scaled up to the surface area of Falls Lake and to annual rates (see Piehler et al. 2020-2021 annual report). The scaled-up rates ranged from 50,000 to nearly 500,000 kg N/y (Figure 12). The average across the three campaigns was 237,000 kg N/y or approximately 47% of the average tributary load of N from 2006 to 2019. This estimate of lake-wide denitrification is approximately 7-fold higher than our estimates based on the nitrogen budget. This discrepancy will be explored further and will likely provide further insights into the nitrogen budget and removal processes in Falls Lake.

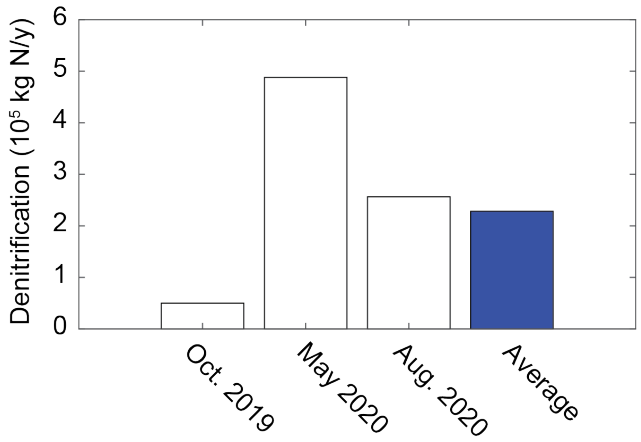

Figure 12. Average denitrifcation rates measured on sediment cores from Falls Lake during three sampling campaigns. Blue bar is the average of all campaigns.

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