Annual Progress Report for Project Entitled

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

Nathan Hall
and
Hans Paerl
7/29/2020

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 in 2020-2021 (Matos, Alix, lead engineer for the B&C model, pers. comm.). During this one year period, 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 N2 fixing cyanobacteria group. Omission of a N2 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 N2 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: A series of five N-fixation assessment campaigns were conducted during the warm months (May-October) between late July 2019 and early July 2020. During each campaign, 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) (Fig 1.). During the first campaign, boat motor troubles resulted in only one of the six stations (station E) being visited but an additional sample was collected approximately 400 m upstream of station E. During May 2020, only the upper five stations were sampled.

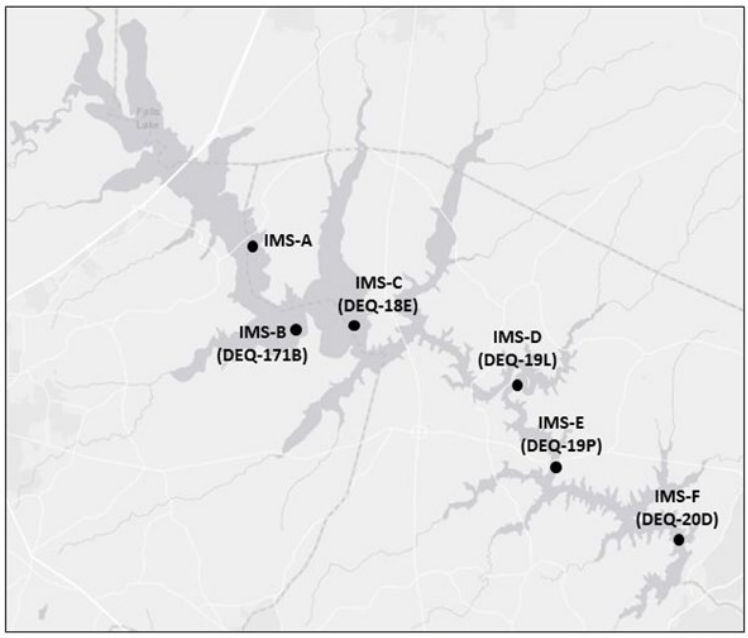

Figure 1. Map of sampling stations for measurements of N fixation rate. Five of the six stations coincided with stations sampled monthly by NC Dept. of Environmental Quality (DEQ).

Measurement of water column N_2 fixation: N_2 fixation was measured using acetylene reduction assays 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 will be 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).

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. In an effort to explore factors that control variability in rates of N_2 fixation, N_2 fixation rates were compared against ambient nutrient concentrations and cyanobacterial biomass determined by HPLC/ ChemTax.

Characterizing the N mass balance: Annual loads of total N and total P for tributaries to Falls Lake and atmospheric deposition of N over the period 2014 to 2018 were taken from the 2019 UNRBA Monitoring Report (UNRBA 2019). 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

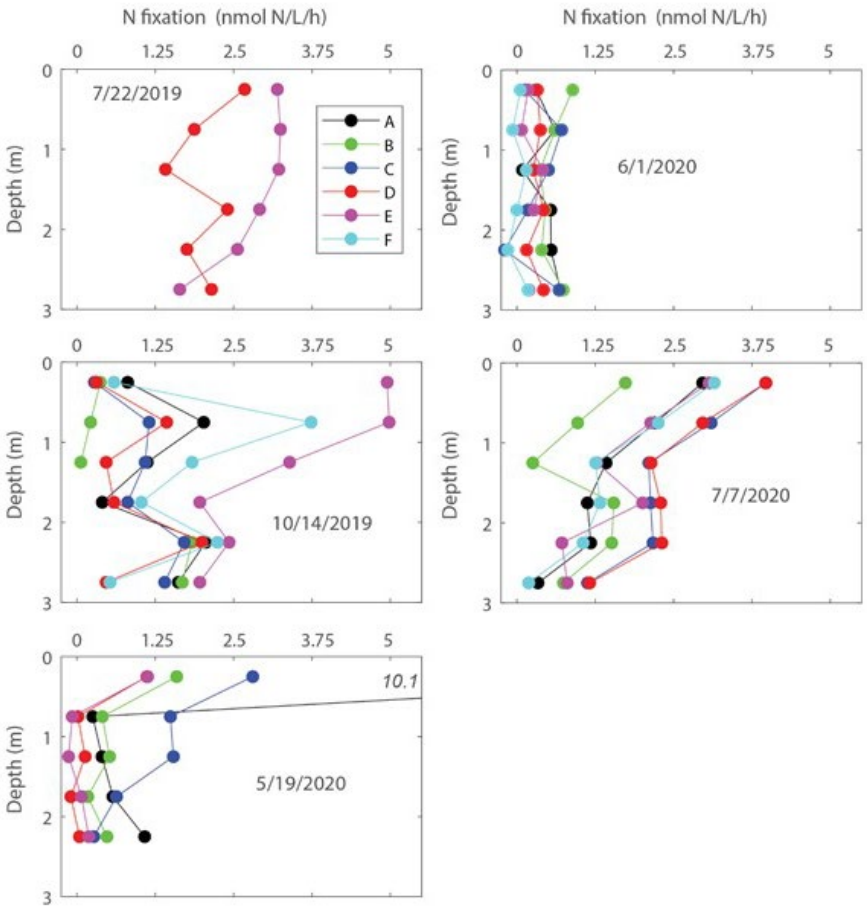

Figure 2. Depth profiles of N fixation measured at six stations (A-F) in Falls Lake during five sampling campaigns during project year 2019-2020.

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 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. However, a Spearman rank correlation between incubation depth and N_2 fixation did not reveal a statistically significant relationship (Rs= -0.12, p = 0.16).

Relationships between depth averaged N_2 fixation and bioavailable, inorganic N and P nutrient forms (nitrate, ammonium, and phosphate), and cyanobacteria biomass estimated by HPLC accessory pigment concentrations were also explored using Spearman's rank correlations to improve understanding of the controls on N_2 fixation in Falls Lake. Maximum N_2 fixation rate within each profile (i.e. the N_2 fixation rate at an optimal irradiance) was also used as the

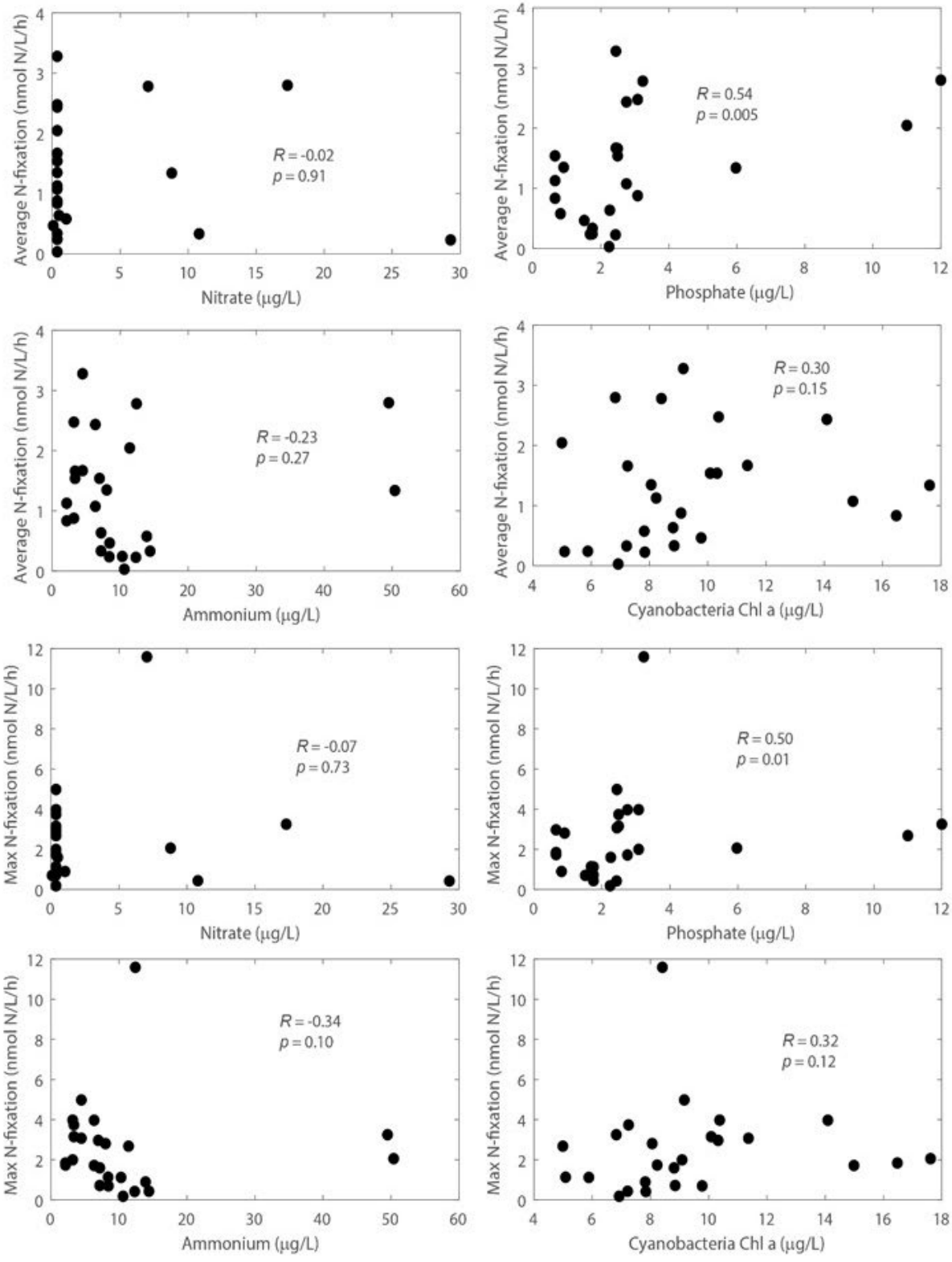

Figure 3. Relationships between inorganic nutrients and cyanobacterial biomass and the depth averaged (top four panels) and maximum (bottom four panels) N fixation rate of each N fixation depth profile measurement. R and p values are results from a Spearman rank correlation.

separate the potential influence of light limitation of N_2 fixation from other factors. Relationships between both depth averaged and maximum N_2 fixation within each profile exhibited no significant relationships to nitrate (Fig. 3). However, for most samples nitrate was below detection, and a value of half the detection limit (0.35 mg/L) was assumed. Stronger, yet still statistically non-significant, negative relationships were observed between ammonium and both average and maximum N_2 fixation. A negative relationship of N_2 fixation with ammonium is expected because elevated ammonium is known to inhibit N_2 fixation (Agawin et al. 2007). Phosphate exhibited a statistically significant relationship to both profile average and profile maximum N_2 fixation. These results are consistent with the current paradigm that N_2 fixation is promoted under conditions of low N and high P availability. The relationship between cyanobacterial biomass determined by HPLC pigment analyses and N_2 fixation was not statistically significant. 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). Microscopic quantification of potentially N_2 fixing, heterocystous taxa in the order *Nostocales* is underway, and comparison of the biomass of heterocystous taxa with rates of N_2 fixation is more likely to reveal a positive relationship with a greater degree of predictive power.

N mass balance: As a first order approximation of the lake wide, annual N input due to N_2 fixation, we simply multiplied the average N_2 fixation value of all measurements across all stations and incubation depths by the total volume of Falls Lake (1.62×10^{11} L) at the top of the conservation pool, lake surface elevation of 251.5 ft), 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). Based on these assumptions used to scale our measurements, the annual N input due to N_2 fixation is ~8460 kg N/ y. Lack of consideration of depth gradients of N_2 fixation likely leads to some error. However, this method for coarsely approximating the lake-wide rate is justified because there was no consistently strong pattern of N_2 fixation in relation to light availability. Future work in 2020-2021, however, will refine this estimate by taking into account observed variation of N_2 fixation with depth and light availability, and the volume represented by each depth strata in the lake.

The annual estimate of N_2 fixation was added to annual estimates of tributary N loads and atmospheric deposition to develop a mass balance for N for the years 2015 to 2018 (Table 1). Over this period, our estimate of annual N_2 fixation represents only 1-2 % of the total tributary flux of TN to Falls Lake. Total N inputs to Falls Lake including tributary inputs, atmospheric deposition, and N_2 fixation ranged from 5.4×10^5 in 2017 to 9.2×10^5 kg N/y during 2018, a very wet year. The percent of total N inputs removed by sedimentation and denitrification in Falls Lake varied from -1% in 2016 to 72% in 2018, and averaged 30%. 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 (NCDWR 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.

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 45 to 89%, and averaging 64%. 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. 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 -1.0×10^5 to 1.8×10^5 kg N/y and averaged 3.6×10^4 kg N/y. In comparison to the total tributary loads of N, denitrification represented between -18 and 28%, and averaged a modest 6% of the total annual tributary loads of N.

Table 1. Mass balance of total N and total P and calculations of lake-wide annual denitrification for the period 2015-2018.

Year	Trib. N load (kg N/y)	Atm. dep. (kg N/y)	N_2 fixation (kg N/y)	All inputs (kg N/y)	River flux out (kg N/y)	TN retained (kg N/y)	% TN retained
2015	6.4×10^5	6.1×10^4	8.5×10^3	7.1×10^5	4.0×10^5	3.1×10^5	56
2016	5.8×10^5	5.9×10^4	8.5×10^3	6.4×10^5	6.4×10^5	0.1×10^5	-1
2017	4.8×10^5	5.4×10^4	8.5×10^3	5.4×10^5	3.4×10^5	1.9×10^5	18
2018	8.6×10^5	[a]5.4×10^4	8.5×10^3	9.2×10^5	5.6×10^5	3.6×10^5	72
Average	6.4×10^5	5.7×10^4	8.5×10^3	6.4×10^5	4.8×10^5	2.2×10^5	30

Year	Trib. P load (kg N/y)	River flux out (kg N/y)	TP retained (kg N/y)	% TP retained
2015	5.8×10^4	2.1×10^4	3.7×10^4	72
2016	6.5×10^4	3.5×10^4	3.0×10^4	45
2017	5.9×10^4	1.9×10^4	4.0×10^4	60
2018	1.2×10^5	2.9×10^4	9.1×10^4	89
Average	7.5×10^4	2.6×10^4	4.9×10^4	64

Year	TN:TP retained	Sediment TN: TP	Denitrif. (kg N/y)	Denitrif. (% Trib. Load)
2015	8.4	3.7	1.8×10^5	28
2016	0.25	3.7	-1.0×10^5	-18
2017	4.9	3.7	4.8×10^4	10
2018	3.9	3.7	2.3×10^4	3
Average	4.4	3.7	3.6×10^4	6

[a]Atmospheric deposition data during 2018 were unavailable and were assumed the same as 2017.

Comparison of mass balance estimates vs. direct measurements of denitrification

The average value of denitrification directly measured by Dr. Piehler's laboratory after scaling to the entire sediment surface area of Falls Lake yielded an annual lake-wide denitrification rate of

4.8×10^4 kg N/y measurements. This value is only slightly higher (33%) than the average value estimated via mass balance, 3.6×10^4 kg N/y. Since, the unusually low N retention in 2016 significantly reduced the average denitrification estimate by mass balance, resolution of the unusual mass balance for 2016 may increase the denitrification rate by mass balance and bring these independent measures of denitrification into even closer agreement. In any case, it appears based on both methods that denitrification removes a modest portion (< 20% on average) of tributary N loads.

References:

Agawin, N.S.R., Rabouille, S., Veldhuis, M.J.W., Servatius, L, Hol, S., Van Overzee, H.M.J., and Huisman, J. 2007. Competition and facilitation between unicellular nitrogen-fixing cyanobacteria and non-nitrogen-fixing phytoplankton species. *Limnology and Oceanography* 52: 2233-2248.

Alperin, M. 2018. Falls Lake Sediment Study. Prepared for the Upper Neuse River Basin Association. UNC Chapel Hill. 23 November 2018.

Grantz, E.M., B.E. Haggard, J.T., Scott. 2014. Stoichiometric imbalance in rates of nitrogen and phosphorus retention, storage, and recycling can perpetuate nitrogen deficiency in highly-productive reservoirs. *Limnology and Oceanography* 59: 2203-2216.

Lin, J., and Li, J., 2011. Nutrient response modeling in Falls of the Neuse Reservoir. *Environmental Management* 47: 398-409.

Molot, L. A., Dillon, P.J.,1993.Nitrogen mass balances and denitrification rates in central Ontario lakes..*Biogeochemistry* 20: 195-212.

NCDWR. 2019. North Carolina Division of Water Resources. Modeling and Assessment Branch. Updated 1990-2018 Loading Estimates for Ambient Stations at Falls Lake Dam, Trent River near Trenton, and Fort Barnwell. Raleigh, NC. October 2019.

Schindler, D.W., Hecky, R.E., Findlay, D.L., Stainton, M.P., Parker, B.R., Paterson, M.J., Beaty, K.G., Lyng, M., and Kaslan, S.E.M., 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proceedings of the National Academy of Science* 105: 11254-11258.

Scott, J.T., M.J. McCarthy. 2010. NitrogeN2 fixation may not balance the nitrogen pool in lakes over timescales relevant to eutrophication management. *Limnology and Oceanography* 55: 1265– 1270.

Touchette, B.W., Burkholder, J.M., Allen, E.H., Alexander, J.L., Kinder, C.A., Brownie, C., James, J., Britton, C.H., 2007. Eutrophication and cyanobacteria blooms in run-of-river impoundments in North Carolina, U.S.A., in: Lake and Reservoir Management. Taylor & Francis Group , pp. 179–192. https://doi.org/10.1080/07438140709353921