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QUANTITATIVE EVALUATION OF CHANGING NUTRIENT SOURCES TO THE ALBEMARLE SOUND SYSTEM

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1. PROJECT GOALS AND OBJECTIVES

Over the last five years, the number of cyanobacteria blooms in the Chowan River and Albemarle Sound reported to NCDEQ has more than doubled what was reported in the early 2000s (Fig. 1). This increase in bloom activity is paralleled by increases in total N and chlorophyll *a* throughout the entire Albemarle Sound system (Fig. 2). The goal of this study was to better understand changes in nutrient sources that fuel the recent increases in cyanobacteria blooms in the Chowan River and Albemarle Sound. At the Albemarle Commission's planning meeting in Edenton on 30 January 2019, several potential nutrient sources were identified as possible causes of recent bloom activity. These included: 1) Loads of N and P from tributary creeks, 2) atmospheric deposition of N, 3) Increased N and P loads due to clear cutting riparian swamp forests, and 4) increased internal N loading due to N fixation. Based on the discussion at that meeting, this project was designed to fulfill the following two objectives.

Objective 1: Quantitatively assess the relative importance of these four potential nutrient sources.

Objective 2: Evaluate the likelihood of increases from each source in causing recent blooms and trends in trophic status in the Chowan River and Albemarle Sound.

Collectively, this information will provide a conceptual framework and establish priorities for research to identify management strategies to restore acceptable water quality.

2. METHODS

The relative importance and potential for causing observed water quality changes for the four source types were assessed using available data sources, published literature, grey literature, and best professional judgement.

2.1 Tributary loads: For tributaries to Albemarle Sound that had gaged stream flow and a long-term record of nutrient concentration data, daily average loads of total N and total P were calculated using the weighted regressions on time, discharge, and season (WRTDS) model (Hirsch et al. 2010) on USGS gaged discharge and monthly concentration data collected by NC DEQ's Ambient Monitoring System (Table 1; Figure 3). The WRTDS program also calculates a flow-normalized load which removes the effect of interannual variability in flow to allow a clearer view of how changes in nutrient sources (or sinks) within the watershed are affecting loads (Hirsch et al. 2010).

Scuppernong R. and Kendrick Cr. that flow into southwestern Albemarle Sound near Mackeys and Columbia, respectively, had long-term records of nutrient concentrations measured by NC DEQ but lacked flow measurements needed to calculate nutrient loads. For those tributaries, time series of estimated stream flows were generated based on measured flows on the Cashie River multiplied by the ratio of the ungaged watershed areas to Cashie River watershed area. This flow estimation method assumes that the water yield (i.e. amount of runoff into stream flow per unit area of watershed) of the ungaged watersheds is equal to the Cashie River. On an annual basis, this assumption is validated based on the similarity of water yields (0.27– 0.35 range) for gaged streams in the region (Table 1). Cashie River was chosen rather than the other streams because it was the smallest of the measured streams and was located closest to the ungaged streams. Watershed areas were accessed through the U.S. EPA Watershed Assessment, Tracking and Environmental Results System's (WATERS) KML application in Google Earth. Nutrient concentrations in Queen Annes Cr. that empties into Edenton Bay (Figure 3) were measured five times during the summer of 2019. With so few data points, a regression approach was unlikely to significantly improve estimated loads. Therefore, annual load was calculated simply as the product of the average measured concentration and annual flow based on the assumption of a water yield equal to the Cashie River (Table 1). To investigate how average loads have changed in relation to the recent increase in bloom activity, average annual tributary loads were calculated for the period 2014-2018 when blooms have been common and for the period 2001-2005 when blooms were less common.

2.2 Atmospheric deposition: Magnitudes and long-term trends of atmospheric N deposition were assessed using data from the two National Atmospheric Deposition Program stations nearest the Chowan River and Albemarle Sound, station NC03 (Figure 3) at Lewiston, NC (36.1325°N, 77.1708°W) and NC06 at Beaufort, NC (34.8846°N, 76.6207°W). Magnitude of N deposition was also assessed using data collected at Pettigrew State Park from 2005-2008 as part of a short-term study designed to test the effect of an industrial poultry operation on N deposition to the Albemarle Sound region (Rossignol et al. 2011). Raw data from station AD3 (Figure 3) in Pettigrew State Park just south of Lake Phelps (35.7373°N, 76.5149°W) were obtained from the lead author, Karen Rossignol. Of the three atmospheric deposition stations used in the study, station AD3 was judged as likely being most representative of deposition over the Albemarle Sound region because it was least impacted from the immediate downwind effects of the poultry operation (Rossignol et al. 2011). Although the published study only included information on rainwater concentrations of nitrate and ammonium, total precipitation amount had been measured and allowed calculation of flux (g N/m^2/week) by multiplying concentration by precipitation amount during each weekly sampling period. Annual fluxes at site AD3 were calculated for the years 2006 and 2007 that had complete weekly records for the entire year. Total N flux to Albemarle Sound in units of 10^6 kg N/y was calculated by summing the weekly fluxes and multiplying by 1.25×10^9 m^2, the surface area of Albemarle Sound (Copeland et al. 1983). As with tributary loads, average annual loads from atmospheric deposition were calculated for the pre-bloom (2001-2005) and current bloom period (2014-2018).

2.3 Nutrient loads from clear cutting swamp forests: In recent years there has been a perceived increase in harvesting of bottomland, hardwood swamp forests driven in part by the wood pellet manufacturing industry (NRDF 2015). Due to the important role that swamp forests play in trapping and removing nutrients from streams that flow to Albemarle Sound (Craig and Kuenzler 1983), possible increases in nutrient loading associated with their harvest deserve consideration. Determining the potential increase in nutrient (N and P) loading associated with swamp forest harvesting requires three pieces of information: 1) the change in nutrient yield (kg N or P/ha/y) associated with forest harvest, 2) the duration of the change in nutrient yield, and 3) the rate of swamp forest harvest in the Albemarle Sound region.

Data on the effects of clear-cut, forest harvesting on N and P yields (kg N or P/ha/y) were gathered from the literature. The duration of the change in nutrient yield was assumed to be one year based on the generally rapid return of nutrient yields determined by the studies and from other literature (e.g. Lebo and Herrmann 1998; Sheperd 1994; Reikerk 1985). Five relevant studies were identified that either directly reported the increase in nutrient yields following clear-cut harvesting or provided enough information to calculate resultant changes in nutrient yields. All five studies were conducted within the coastal plain of the southeast US and three of the studies (Ensign and Mallin 2001, Grace 2004, and Lebo and Hermann 1998) were conducted in North Carolina. Two of the studies (Ensign and Mallin 2001, Grace 2004) were conducted on alluvial, hardwood, swamp forests very similar to those being harvested in the Albemarle Sound region and the other three (Lebo and Hermann 1998, Reikerk 1983, and Wynn et al. 2000) were conducted in pine silviculture operations. For studies where nutrient yields were calculated for periods other than an annual period, yields were recalculated to an annual basis. The Ensign and Mallin (2001), Grace (2004), and Reikerk (1983) studies did not specifically report the increased nutrient yield following harvest. For those studies that contained a before/after/control/impact (BACI) design, the increased yield due to harvesting was calculated as the difference between post-harvest and pre-harvest yields less the change in nutrient yield that occurred over the same period in the control watershed (Smokorowski and Randall 2017).

Values of TN and TP concentration change following swamp forest harvest were reported by Ensign and Mallin (2001), but the effect on TN and TP load was not reported. Since flow out of Goshen Swamp is not gauged, stream flow was estimated by scaling North East Cape Fear River flows (USGS 02108000, the nearest gauged watershed and includes Goshen Swamp) by the ratio of Goshen Swamp to North East Cape Fear River watershed area. Nutrient yields for TN and TP were then determined as the loads divided by the 52 hectare harvested area and resulted in increased yields of 2700 kg N/ha/y and 350 kg P/ha. These values are two orders of magnitude higher than increased yields determined from other studies, and caused us to question the validity of the assumption that the increased yields truly reflected the impacts of the swamp forest harvest. The portion of Goshen Swamp that was harvested represented only 0.1% of the total Goshen Swamp watershed (52 of 47,900 ha). Under the assumption of equivalent water yield of the North East Cape Fear River, the total Goshen Swamp watershed, and the harvested portion of Goshen Swamp, the observed increases in TN and TP yields from the harvested area would have required that the TN and TP concentrations in the flows delivered from the harvested area would have to be 801 and 106 mg/L, respectively. Considering that these values are at least an order of magnitude higher than TN and TP concentrations of raw sewage (Tchobanoglous et al. 2003), it seems highly unlikely that waters of such high concentrations emanated from a swamp forest. A more likely scenario is that, coincidentally with the swamp forest harvesting, a substantial increase of TN and TP inputs occurred from a large, undocumented source upstream of the harvested region in Goshen Swamp. For this reason, we decided not to use the results from the Ensign and Mallin (2001) study in determining potential increases in loading to Albemarle Sound from swamp forest harvesting.

The area of swamp forest harvested annually in the Albemarle Sound region was estimated based on reports published by the Southern Environmental Law Center (SELC 2015), RTI International (RTI 2016), the NC Forestry Service (Lorber and Rose 2015), and the US Forest Service's (USFS) Forest Inventory Analysis (FIA) database. The USFS-FIA program is a sample-based approach involving periodic quantification of forestry activity at designated forestry plots throughout the U.S. The FIA purpose is to quantify national to large scale regional trends in the status of US forests. As trends at finer spatial scales are explored, the standard error in estimates of harvested acreage increases and range up to about fifty percent of harvest estimates for the sum of all counties adjacent to Albemarle Sound (Lorber and Rose 2015).

The RTI report contained data from the Forest Inventory Analysis Timber Products Output (FIA-TPO) program including the annual average total cubic feet of wood harvested from each county in North Carolina and Virginia that was within the Albemarle/ Pamlico Sound watershed over the period 2002-2012. Assuming a harvest yield of 8250 cubic feet of wood per hectare (RTI 2016), allowed conversion of harvests in units of wood produced to harvested acres. Total harvested acreage was divided by three to account for the two thirds of forest harvests that were from pine silviculture rather than bottomland hardwood. Harvests were summed over North

Carolina and Virginia counties that were within the Albemarle Sound watershed and that would contribute directly to the sound without any increased loads being assessed as an increase in tributary loading. These counties included (North Carolina counties): Bertie, Camden, Chowan, Currituck, Dare, Gates, Halifax, Hertford, Hyde, Pasquotank, Perquimans, Tyrell, Washington, and (Virginia counties) Southampton, Suffolk, Chesapeake, and Virginia Beach. Estimates of bottomland hardwood from the SELC and RTI reports were additionally compared to the most recent (2018) USFS FIA data for the combined Oak-Gum-Cypress and Ash-Elm-Cottonwood forest groups that dominate swamp forests of the Albemarle Sound region.

2.4 Internal N loads due to N_2 fixation: Nitrogen input due to nitrogen fixation was estimated using acetylene reduction assays on water samples collected from the Chowan River and its tributaries by the Chowan/ Edenton Environmental Group. On five dates spanning mid-June to mid-October 2019, water samples were collected from 4 to 7 stations (stations 1, 2, 4, 6, 8, 10, and 11) throughout the Chowan River (Figure 3). On four dates from late July to October, samples were also collected at a station on Queen Anne's Creek that flows into Edenton Bay. On 30 August 2019, a sample was collected from Indian Creek, and on 12 September 2019, samples were collected at five stations on Potecasi Creek (Figure 3). Water samples were collected approximately at midday and shipped overnight to the UNC-IMS in insulated coolers to maintain *in situ* temperature conditions.

From each sample, triplicate 50 mL aliquots of sample water were dispensed into 85 mL borosilicate glass serum vials and stoppered with a rubber septum stopper. Three mL of headspace was evacuated from each vial and was immediately replaced with 3 mL acetylene gas. The acetylene injected samples were then incubated for approximately three hours (~11:00-14:00) under natural lighting and temperature conditions in flowing seawater ponds behind UNC-IMS. The bottles were shaded with two layers of neutral density screening to prevent photoinhibition caused by damaging near surface irradiance and to more accurately simulate the lower light levels that are typical in the turbid and stained waters of the Chowan River. The shading resulted in a light level that was about 25% of incident irradiance.

After the incubation, the vials were shaken vigorously for 30 s to ensure equilibration between dissolved gases and the head space. Three mL of headspace was removed from each vial using a syringe and injected into a pre-evacuated three mL Vacutainer (Becton Dickinson

Inc.) tube. Aliquots of 0.2 mL of gas samples were measured for ethylene and acetylene by flame ionization gas chromatography using a Shimadzu GC9 Gas Chromatograph. Rates of N_2 reduction to ammonia were estimated from acetylene reduction to ethylene by dividing the acetylene reduction rate by 4 (Paerl et al. 2014). A triplicated blank of deionized water was used on each incubation date to account for any non-biological reduction of acetylene. Average blank values were then subtracted from the values of sample water to determine the biological rate of N_2 reduction.

Average values of N_2 fixation from samples collected in the Chowan River were used to estimate the contribution of N_2 fixation to the total N budget of the whole Albemarle Sound system. Rates of N_2 fixation in units of μg N/L/h that were measured during the acetylene reduction assays were scaled up to a Albemarle Sound rate in units of 10^6 kg N/y by first multiplying by 6.5×10^{12} L, the volume of Albemarle Sound (Copeland et al. 1983). Average hourly rates measured at midday were multiplied by a 12 hour photoperiod to estimate daily N_2 fixation and the daily average was then multiplied by 180 days to estimate the annual rate based on the assumption that N_2 fixation would be quantitatively important only during the warmer half of the year (Piehler et al. 2009).

In addition to direct measures of N fixation that were made during summer 2019, a long-term record of N_2 fixation potential was constructed based on the biomass of N_2 fixing cyanobacteria measured by NC DEQ at four Ambient Monitoring System stations. Stations D8950000 and D9490000 were respectively located at Colerain and the HWY 17 bridge on the Chowan River, and stations M610000C and M390000C were respectively located in the mid and eastern, central areas of Albemarle Sound (Figure 3). For each species, cell density (cells per mL) and biomass as biovolume (cubic micrometers of algae per liter of sample) were provided. 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. The heterocystous genera identified were *Anabaena*, *Anabaenopsis, Aphanizomenon, Cylindrospermopsis, Dolichospermum,* and *Raphidiopsis*. The nitrogen content of N fixing cyanobacteria was calculated by multiplying biovolume by 2.8×10^{-15} mol/ μm^3. A review of N_2 fixation rates of natural assemblages of heterocystous cyanobacteria (Klawonn et al. 2016), found average N specific N_2 fixation rates of 0.01 mol N/ mol N/h with a 90% confidence interval that spanned from 0.001 to 0.042 mol N/ mol N/h. The annual average N_2-fixing cyanobacterial N content was multiplied by 0.01 mol N/ mol N/h to determine an annual average hourly rate per liter of water which was subsequently scaled up by multiplying by a 12 hour photoperiod, 180 day growing season, and the 6.5×10^{12} L volume of Albemarle Sound. This same process was repeated using the upper and lower bounds of the 90% confidence interval of the literature derived N-specific N_2 fixation rate to convey uncertainty in the actual, unmeasured N-fixation activity of the heterocystous cyanobacteria. Average annual values across all four stations were used as a metric of system-wide changes in the potential importance of N_2 fixation over time and to compare with N loads from tributaries, atmospheric deposition, and potential inputs resulting from swamp forest harvesting.

3. RESULTS

3.1 Tributary Loads: Nutrient loading records for total N and total P were established for the Roanoke R., Chowan R., Potecasi Cr., Cashie R., Scuppernong R., and Kendrick Cr. from the mid 1980's through 2018 (Figures 4 and 5). Compared to the relatively bloom-free period (2001-2005), average TN loading increased by 11 to 100 percent in seven of the eight assessed tributaries during the recent bloom period (2014-2018) (Table 2). Because the Roanoke R. constitutes such a large portion of flow to Albemarle Sound, the 31% increase in TN loading from the Roanoke river constituted about 80% of the total increase in TN load from the eight tributaries that were assessed. The Chowan River showed a small, 10%, reduction in TN loading but this decrease had only a small, ~ 5%, impact on the combined TN loading of the six tributaries. As with TN, between the two five-year periods average TP loading also showed a general increase of about 10 to 50 percent among all station except the Chowan River (Table 2). The decline in loading from the Chowan River nearly matched the combined increases from the other tributaries such that TP loading of these six tributaries has exhibited little change between the pre-bloom and bloom period.

Much of the interannual variability in TN and TP loading is due to the interannual variation in stream flow. For example, all the streams show elevated TN and TP loads during the very wet year of 2003, and reduced loads during the drought year of 2007. High flows during 2003 were largely responsible for the higher observed TN and TP loads in the pre-bloom period on the Chowan R. (Figures 4 and 5).

The WRTDS analysis packaged that was used to calculate loads also calculates a flow-normalized load which removes the effect of interannual variability in flow to allow a clearer view of how changes in nutrient sources (or sinks) within the watershed are affecting loads (Hirsch et al. 2010). Between the pre-bloom period and current bloom-period, the five-year average flow normalized TN load increased in all the assessed tributaries except the Perquimans River which exhibited a slight (3 %) decrease in TN load (Table 2). Flow normalization removed the strong influence of 2003 loading in the Chowan record and revealed a general increase in TN inputs to the Chowan. For the other tributaries, flow normalization removed the effect of the high flow years of 2017 and 2018 in the recent bloom period. Consequently, for these stations flow normalized TN loads did not increase as much as the actual loads at these stations (Table 2). However, the increases in flow normalized loading were still substantial, 19-22%, for the three largest tributaries assessed and demonstrate increases in TN inputs to these major tributaries that cannot solely be explained by interannual variation in precipitation. For TP, the impact of removing the influence of the recent high flow years resulted in all the streams except Kendrick Cr. showing essentially no change or a decrease in TP inputs (Figure 5, Table 2). Kendrick Cr. still showed a 34% increase in TP that indicates some increasing TP sources in that watershed between the two five-year periods (Table 2).

Total freshwater inflows to Albemarle Sound average about 15,000 × 10^6 m^3/y (Copeland et al. 1983), and the flows of the ten streams assessed in this report add up to ~13,000 × 10^6 m^3/y (Table 1). So, about 15% of the tributary inflows to the Albemarle Sound is occurring through smaller, coastal streams with ungaged flows and unknown concentrations, and whose proximity to the Sound may reduce the opportunity for instream attenuation between nutrient sources and the Sound. TN concentrations in the coastal streams ranged from 1.03 – 2.79 mg/L compared to 0.6 to 0.8 mg/L for the major rivers (Table 2) and TP also showed a strong tendency toward elevated values in the coastal streams (Table 2). If we assume that the average nutrient concentrations of the unassessed streams are similar to the smaller, coastal plain tributaries that were assessed, then the TN and TP loads from the unassessed streams would be 4.2 × 10^6 kg N/y and 0.37 × 10^6 kg P/y for the period 2014-2018 (Table 3). Although they only carry about fifteen percent of the flow, the unassessed streams could carry up to 40% of the N and P load (Table 3). In contrast, if the unassessed streams have concentrations similar to the major rivers, then their contribution to total stream loads of N and P is only about 13% (Table 3). A recent analysis of potential nutrient loading using the Natural Capital Project's Integrated Valuation of Ecosystem Services and Tradeoffs (InVEST) model identified many of these small, unassessed coastal plain watersheds as having very high yields (Hillman et al. 2019). Clearly, more work is needed to assess the magnitudes and begin to measure any changes that may be occurring in the nutrient loads of these small, coastal streams.

3.2 Atmospheric Deposition: The NADP station at Lewiston, NC is the closest station with a long-term record of nitrogen atmospheric deposition to the Chowan R. and Albemarle Sound (Figure 3). The NC03 record shows a clear pattern of decreasing nitrate deposition from around 2 kg N/ha/y prior to 2000 to about 1 kg N/ha/y by the end of the record in 2018. The same declining trend and similar annual fluxes of nitrate were also measured at NADP site NC06. Nitrate measurements in 2006 and 2007 near Lake Phelps were also very similar to the values at the NADP sites. This declining trend in nitrate is a national trend and almost certainly occurred throughout the Chowan R./ Albemarle Sound region.

In contrast to nitrate, in the past twenty years ammonium has increased by 1 kg N/ha/y from about 1.5 kg N/ha/y to 2.5 kg N/ha/y. The increasing trend in ammonium is apparent at both NC03 and NC06. However, fluxes were about 0.5 kg N/ha/y higher at station NC03 and at Lake Phelps compared to station NC06. Based on the greater similarity between the NC03 and Lake Phelps ammonium records and their greater proximity to Albemarle Sound, the NC03 record was used to infer the history of atmospheric deposition to Albemarle Sound. With a similar magnitude of increase in ammonium as the decrease in nitrate, total atmospheric flux of inorganic nitrogen to the region has changed very little of the past forty years and averages about 3.5 kg N/ha/y. Scaled to the 125,000 hectare surface area of the Albemarle Sound ecosystem, atmospheric deposition contributes about 0.45×10^{6} kg N/y to the N flux of the system (Table 3).

In addition to nitrate and ammonium, total dissolved nitrogen was also measured at site AD3 which allowed calculating dissolved organic nitrogen deposition by subtracting the nitrate and ammonium from total dissolved nitrogen. On average, only 5% of the total dissolved nitrogen was in organic forms. This indicates that the NADP sites which do not measure organic nitrogen are likely not missing a major fraction of the atmospheric N load.

3.3 Swamp Forest Harvest: The Southern Environmental Law Center (SELC) estimated that operating the three currently existing Enviva wood pellet manufacturing facilities plus two planned facilities would require harvesting 7800 hectares of bottomland hardwood forest per year (SELC 2014). Assuming the two new plants will require a similar amount of material as the three existing facilities, the current harvest is estimated at 4600 hectares per year. The estimate of annual swamp forest harvest, based on RTI's report (RTI 2016) on timber output from the counties surrounding Albemarle Sound, was nearly identical at 4580 hectare per year (Table 4). Personnel with the NC Forestry Service believe that 4600 hectares per year is an overestimate (Tom Gerow, Water Resources Staff Forester, NC Forestry Service pers. comm.), and point out that Enviva has recently committed to reducing their utilization of hardwood and will produce pellets that are up to 90% pine (Barry New, Technical Development & Planning Branch Head, NC Forestry Service, pers. comm.). Analysis of a report of FIA data generated using EVALIDator Version 1.8.0.01 from 2018 for the North Carolina counties listed in Table 4 showed an annual harvest of only 1770 (+/- 1350 S.E.) hectares of swamp forest (oak-gum-cypress & ash, elm, cottonwood) but with a large degree of uncertainty. Acknowledging that 4600 hectare per year may overestimate the swamp forest harvest rate, the estimate of 4600 hectare per year harvest can still provide a valuable worst-case scenario to constrain the upper range of potential nutrient load increases due to swamp forest harvesting.

Estimates of the increased yield of TN resulting from the harvest of a hectare of swamp forest versus pre-harvest yields varied among the studies that were reviewed by a factor of 40 from ~2 to 80 kg N/ha/y (Table 5), and depended heavily on the specific forestry practices employed during the harvest, e.g. implementation of best management practices (Wynn et al. 2000; Reikerk 1983). Increased yields of TP from clear cut swamp forests varied from negative values (clear cut retains slightly more TP than prior to harvest) to a TP yield of 11 kg P/ha/y (Table 5). After scaling up these estimates of changes in yield to the area of swamp forest harvested annually, we estimated that swamp forest harvest results in annual TN loading to Albemarle Sound ranging from about 0.01 to 0.4×10^6 kg N/y and changes in TP loading ranging from -0.0003 to 0.05×10^6 kg P/y (Table 5).

The above values of post-harvest increases in nutrient yields do not indicate that this is the amount that loading from swamp forest harvesting has increased over time. Since the period of enhanced nutrient loading following harvest is short-lived (Shepherd 1994), there would also need to be an increase in the rate of swamp forest harvesting to produce an increasing trend over time in the loading attributable to harvesting swamp forest. A comparison of FIA data from the Albemarle Sound region for the five year periods 2002-2007 and 2007-2012 indicated that the wood harvest volume from bottomland forests decreased from 77×10^6 ft^3/y to 12×10^6 ft^3/y, and bottomland forest area increased by 5,900 ha/y, including a 2,700 ha/y increase in the oak-gum-cypress category that dominates the wettest, riparian swamp forests (Lorber and Rose 2015). A more recent unpublished study, however, did determine that on a statewide basis there has been a net annual loss of 1200 ha/y over the ten year period from 2006 to 2016 (Brown 2017). The fraction of this statewide loss that occurred in the Albemarle Sound region was not reported. Based on the lack of evidence for a significant increase, and the possibility that there has been a recent decrease in the rate of swamp forest harvest in the Albemarle region, it seems unlikely that the trend in loading from this source has increased significantly over the period that the algal bloom problem has worsened.

3.4 Cyanobacterial Nitrogen Fixation: Phytoplankton community composition data from NCDEQ show a clear increase in the proportion of the phytoplankton community that is composed of potentially N_2-fixing, heterocystous cyanobacteria (Figure 6). For both the Chowan River sites and Albemarle Sound, the heterocystous cyanobacteria fraction of the community increased from being a negligible fraction (< 5 %) from 2000 to 2004 to comprising on average about 30% (range 0-90) of the community from 2014-2018 (Figure 7).

Assigning a literature value of biomass specific N_2 fixation to the heterocystous cyanobacteria enabled estimation of a time series of potential N_2 fixation by the cyanobacteria community. The results demonstrate that the change in heterocystous cyanobacteria biomass has been sufficient to increase N_2 fixation by two orders of magnitude over the eighteen-year data record. Based on an average value of N_2 fixation per unit biomass, N loading to Albemarle Sound due to N_2 fixation for the recent bloom period (2014-2018) could be as high as 8.2×10^6 kg N/y (Figure 8, Table 3). This value is approximately the same as the sum of all tributary inputs and indicates that increases in N_2 fixation could certainly play a prominent role in the observed changes in trophic status within Albemarle Sound.

The presence of high biomass levels of heterocystous cyanobacteria provides evidence but is not proof of the importance of N_2 fixation. It is possible to have high biomass levels of heterocystous cyanobacteria and yet low levels of N_2 fixation (Piehler et al. 2009). This appears to be the case for the Chowan River based on the N_2 fixation measurements during summer 2019. For each of the eight stations that were sampled 4 to 5 times during summer of 2019, median N_2 fixation rates for each station measured by acetylene reduction were less than 7.5 nmol/L/h and when scaled to the growing season and volume of Albemarle Sound were less than 0.25×10^6 kg N/y (Figure 9). Similarly-low values were also measured on one date at five stations on Potecasi Creek, and on one date and one station on Indian Cr. No strong spatial patterns were apparent among the Chowan River stations. Values as high as 42 nmol/L/h were measured (Figure 9) and the few higher values elevated the average of all the measurements to 6.9 nmol/L/h or 0.23×10^6 kg N/y when scaled to an annual rate for Albemarle Sound (Table 3).

Given the low measured N_2 fixation rates but recent history of substantial, heterocystous cyanobacteria biomass, we sought to determine whether the low measured N_2 fixation rates were due to an unusual absence of heterocystous cyanobacteria among the 2019 samples, or instead due to low activity of the heterocystous cyanobacteria biomass present. To address this question, biovolume of heterocystous cyanobacteria (μm^3/L) was quantified microscopically for eleven samples collected on 30 August 2019. On this date, measured N_2 fixation rates spanned 0 to 36 nmol/L/h, nearly the entire range of observed rates for the whole summer. The N content of the heterocystous cyanobacteria per unit of biovolume was estimated as 2.8×10^{-15} mol N/μm^3. Dividing the measured N_2 fixation rate by the N concentration of the heterocystous cyanobacteria provided an estimate of the N-specific N_2-fixation activity of the heterocystous cyanobacteria. These values were compared against the literature values from the review by Klawonn et al. (2016) that were used to generate the time series of potential N_2 fixation from the NCDEQ phytoplankton cell count data. Results demonstrated that the measured activity was in fact very low for the amount of heterocystous biomass present in the Chowan River on 30 August 2019 (Figure 9). At all but two stations, rates were lower than the 10^{th} quantile of the data reviewed by Klawonn et al. (2016) and the highest value at station 6 was only a quarter of the median rate of heterocystous cyanobacterial N_2 fixation rates reviewed by Klawonn et al. (2016) (Figure 10).

4. CONCLUSIONS AND NEXT STEPS

The combined Roanoke, Chowan, and Meherrin Rivers constitute between 55 to 70% of the N and P loads to Albemarle Sound, with the remainder of the load and the high degree of uncertainty due to the inputs of smaller coastal plain streams. Given the high concentrations and more rapid increases in total N loads from the coastal plain streams that were assessed, and the fact that such streams constitute about a quarter of the total watershed of Albemarle Sound, it is possible that the smaller, and largely unassessed, coastal plain streams contribute up to forty five percent of N and P loads to Albemarle Sound. Potecasi Creek and the Cashie River, in particular, have experienced significant increases in TN loading over the past fifteen years, and atmospheric concentrations of ammonium are increasing in this same area. Understanding the land use changes behind these trends is an important topic for further research.

Improved quantification of the contributions of these smaller, coastal plain streams to changes the nutrient loading of Albemarle Sound should be a priority for future research. Both stream flow and nutrient concentration data are necessary to calculate loads. Nutrient concentrations are assessed for only about a third of the flows delivered by coastal plain streams, and stream flows are only gaged for two of the coastal plain streams, Potecasi Creek and Cashie River. Although stream flows are only measured for two of the coastal plain streams, the relatively constant water yield of watersheds that were gaged (Table 1) indicates that an assumption of a constant water yield across Albemarle Sound's coastal watersheds may not lead to serious errors in calculated loads. The highly variable nutrient concentrations of coastal plain tributaries (Table 2), however, indicates that making assumptions about concentration may lead to significant errors in calculating loads. While having more information on stream flows and concentration would be desirable, focusing scant resources on gathering more information on nutrient concentrations in the coastal plain streams will likely yield the most cost-effective reductions in uncertainty for nutrient loading to Albemarle Sound.

The trend in elevated algal productivity that has precipitated the current harmful algal bloom problem is likely associated with the long-term trend of increasing total N concentration with a slope of about 0.01- 0.02 mg/L/y. For the Albemarle Sound system that has a volume of 6.5 ×

10^{12} L, this represents an annual increase of 0.06 - 0.13 × 10^6 kg N/y. From a mass balance perspective, any increase in source greater than this amount could be responsible for the observed declines in water quality. Considering that a source responsible for the increase in TN concentration must exceed 0.06 - 0.13 × 10^6 kg N/y rules out atmospheric deposition as a potential cause; rather unsatisfying since the trend in atmospheric deposition was a declining trend anyway. In comparison to tributary inputs, atmospheric deposition is also a minor contributor (2 - 4 % depending on uncertainties in loading from unassessed tributaries and N fixation) to the total N flux to Albemarle Sound.

The small measured rate of cyanobacterial N fixation during summer 2019 (0.23 × 10^6 kg N/y) is just large enough. Potential N fixation estimates based on the biomass of heterocystous cyanobacteria during the recent bloom period were nearly two orders of magnitude higher than measured rates and were of similar magnitude to the rates of N loading from all the tributaries combined (Table 3). If only a fraction of the N fixation potential were realized, then increases in cyanobacterial N fixation could simultaneously play the major role in causing observed increases of TN and phytoplankton biomass.

There is an increasing recognition that rates of microbially mediated biogeochemical processes are highly variable through space and time. Long periods and/or vast expanses of low rates are punctuated by “hot spots” and “hot moments” of high rates that can account for the vast majority of material processing when integrated over broad spatial and temporal scales (McClain et al. 2003). It is possible that the sampling effort, total of five dates and about ten locations, missed (either spatially or temporally) these hot spots, and that the rate of N_2 fixation is actually more significant than the current sampling effort has indicated. In such cases where bursts of activity can be quantitatively important but difficult to directly measure, approaches that integrate the results of the activity over large spatial or temporal scales can be useful for determining the importance of a microbial process (McClain et al. 2003). In the case of N_2 fixation, stable nitrogen isotope analysis that takes advantage of the isotopically light signature of microbially fixed N could provide a powerful, spatially and temporally integrative tool to assess the importance of N fixation for the N budget of Albemarle Sound (Holl et al. 2007).

Swamp forest harvest seems unlikely to be a major contributor to the observed changes in water quality in Albemarle Sound. Even using the highest estimates for increased TN yield and what is likely an overestimate of the harvest rate, the increased N load would only amount to about 5% of the assessed tributary load. However, using the worst-case yield and harvest scenarios, swamp forest harvest cannot be completely ruled out as a potential cause. Additionally, this project sought to determine the importance of swamp forest harvesting on an Albemarle Sound-wide scale. If harvesting is concentrated within particular watersheds then there may be larger relative increases in nutrient loading for those watersheds than indicated by this report. More studies of increased yield resulting from swamp forest harvest that are conducted within the Albemarle Sound region, and a better accounting of the harvest rate are needed to refine estimates of the impact of swamp forest harvesting on nutrient loading to Albemarle Sound.

The total flux of TN through Albemarle Sound is likely to be at least 100 times greater than the rate that TN is accumulating with its waters. In addition to inputs and changes in concentration, the mass balance of TN in estuaries is also highly impacted by changes in loss terms (Howarth 1988). It is definitely possible that the major driver of increasing levels of TN is not increasing inputs at all, but rather is a decrease in the rate TN is removed from the system. In particular, rates of denitrification along the margins of Albemarle Sound may be changing as a result of sea level rise. The substantial wetlands that rim Albemarle Sound undergo seasonal bursts of summertime denitrification. The timing is partly due to warmer sediment temperature but the primary driver of the seasonality is the summertime draw down of the surface water table that results from evapotranspiration. Lower summer water tables allow oxygen to penetrate the sediments and allow ammonium that has accumulated during inundation to be rapidly nitrified to nitrate (Brinson et al. 1978). If rising sea levels prevent the annual draw down of the water table, rates of riparian denitrification could be reduced and result in increases in N concentrations within Albemarle Sound. A greater understanding of the role of denitrification within the Albemarle Sound system, and how it is likely to responding to local sea level rise is another important avenue for future research.

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Table 1. Summary of data sources and methods used to calculate tributary loads to the Albemarle Sound.

Tributary	Flow Data Source	Concentration Data Source	Load Calculation Method	Watershed Area (km^2)	Average Flow (10^6 m^3/y)	Water Yield (m/y)
Roanoke R.	USGS 02080500	NC DEQ station N8550000	WRTDS[a] measured flow & concentrations	24,472[b] (21,714)	7,720	0.31
Chowan R.	Nottoway R. (USGS 02047000) + Blackwater R. (USGS 02050000)	NC DEQ station D0010000	WRTDS measured flow & concentrations	6,380 (6,177)	1,760	0.29
Meherrin R.	USGS 02052000	NC DEQ station D5000000	WRTDS measured flow & concentrations	4,176 (1,927)	1,338	0.32
Cashie R.	USGS 0208111310	NC DEQ station N8950000	WRTDS measured flow & concentrations	825 (279)	97	0.35
Potecasi Cr.	USGS 02053200	NC DEQ station D4150000	WRTDS measured flow & concentrations	669 (583)	160	0.28
Perquimans R.	Estimated from Cashie R. flow	NC DEQ station M500000	WRTDS estimated flow & measured concentrations	562	182 [c]	0.32[d]
Scuppernong R.	Estimated from Cashie R. flow	NC DEQ station M6980000	WRTDS estimated flow & measured concentrations	466	149[c]	0.32[d]
Little R.	Estimated from Cashie R. flow	NC DEQ station M3500000	WRTDS estimated flow & measured concentrations	344	112 [c]	0.32[d]
Kendrick Cr.	Estimated from Cashie R. flow	NC DEQ station M6920000	WRTDS estimated flow & measured concentrations	279	90[c]	0.32[d]
Queen Annes Cr.	Estimated from Cashie R. flow	Albemarle Commission	Average concentration × annual flow	19	6.1 [c]	0.32[d]
Albemarle Sound	Giese et al. (1979)	--	--	46,600	15,000[e]	0.32

[a]Weighted regression on time discharge and season (Hirsch et al. 2010). [b]Value does not include Cashie River watershed area. [c]Value calculated using an assumed water yield of [d]0.32 based on measured water yields of proximal watersheds. [e]Value calculated by Giese et al. 1979.

Table 2. Average tributary load estimates for total N and total P during a period before (2001-2005) and within (2014-2018) the recent period of enhanced cyanobacteria bloom activity in the Albemarle Sound region.

	Time Period	Roa.	Chow.	Meh.	Cash.	Pot.	Perq.	Scup.	Litt.	Ken.	[a]QAC
Actual Loads											
TN (10^6 kg N/y)	2001-2005	4.29	1.45	0.80	0.30	0.19	0.31	0.38	0.22	0.13	--
	2014-2018	5.63	1.31	0.65	0.61	0.29	0.34	0.51	0.27	0.18	0.008
Δ (10^6 kg N/y)		1.34	-0.14	-0.15	0.31	0.10	0.03	0.13	0.05	0.05	--
Δ (%)		31	-10	-19	105	51	9	34	21	41	--
TP (10^6 kg P/y)	2001-2005	0.45	0.17	0.08	0.05	0.03	0.03	0.02	0.03	0.005	--
	2014-2018	0.49	0.11	0.06	0.06	0.03	0.02	0.03	0.04	0.007	0.002
Δ (10^6 kg P/y)		0.04	-0.06	-0.02	0.01	0.01	-0.06	0.01	0.06	0.002	--
Δ (%)		8.7	-33	-28	19	1.8	-19	40	20	51	--
[b]Flow Weighted Concentrations											
TN (mg N/L)	2001-2005	0.51	0.69	0.58	1.00	0.86	1.67	2.47	1.92	1.36	--
	2014-2018	0.60	0.80	0.69	1.73	1.03	1.53	2.79	1.96	1.62	1.25
Δ (mg N/L)		0.09	0.11	0.12	0.73	0.17	-0.13	0.33	0.04	0.26	--
Δ (%)		0.18	0.15	0.20	0.73	0.19	-0.08	0.13	0.02	0.19	--
TP (mg P/L)	2001-2005	0.06	0.08	0.06	0.17	0.14	0.16	0.15	0.28	0.05	--
	2014-2018	0.06	0.07	0.06	0.17	0.11	0.11	0.18	0.28	0.07	0.29
Δ (mg P/L)		0.00	-0.01	0.00	0.00	-0.03	-0.05	0.03	0.00	0.01	--
Δ (%)		-2	-14	6	0	-19	-31	18	1	27	--
Flow Normalized Loads											
TN (10^6 kg N/y)	2001-2005	4.11	1.23	0.83	0.27	0.18	0.30	0.39	0.21	0.13	--
	2014-2018	4.90	1.44	1.07	0.47	0.23	0.29	0.40	0.23	0.14	--
Δ (10^6 kg N/y)		0.79	0.21	0.24	0.2	0.05	-0.01	0.01	0.02	0.01	--
Δ (%)		19	17	29	74	28	-3	2	9	8	--
TP (10^6 kg P/y)	2001-2005	0.49	0.14	0.04	0.05	0.03	0.03	0.03	0.03	0.01	--
	2014-2018	0.49	0.13	0.04	0.04	0.02	0.02	0.02	0.03	0.01	--
Δ (10^6 kg P/y)		0.00	-0.02	0.00	-0.00	-0.01	-0.01	-0.00	0.00	0.00	--
Δ (%)		1	-14	7	-6	-22	-24	-5	0	34	--

[a]Nutrient loads for Queen Anne Creek were based on measurements made in 2019 but are assumed representative of loads during the recent, 2014-2018, period. Roa. = Roanoke River. Chow = Chowan River, Meh. = Meherrin River, Pot. = Potecasi Creek, Perq. = Perquimans River, Scup. = Scuppernong River, Litt. = Little River, Cash. = Cashie River, Ken. = Kendrick Creek, QAC = Queen Annes Creek. [b]Flow weighted concentration was calculated as the annual load divided by the annual flow.

Table 3. Comparison of total N and total P loads from tributaries, atmospheric deposition, N fixation, and swamp forest harvesting during the period prior to (2001-2005) and since (2014-2018) the recent increase in cyanobacteria bloom activity in the Albemarle Sound region.

	Large rivers[a]	Smaller assessed tributaries[b]	Unassessed tributaries[c]	Atmospheric Deposition	N_2 fixation potential (measured)	Swamp forest harvesting
Total N load (10^6 kg N/y)						
2001-2005	6.54	1.53	1.12 – 2.92	0.52	0.78 (---[d])	---
2014-2018	7.59	2.19	1.30– 4.18	0.45	8.20 (0.23)	0.01 – 0.4
Change	1.05	0.66	0.18 – 2.71	-0.07	7.42 (---)	---
% Change	16	43	16 – 43	-13	951 (---)	---
Total P load (10^6 kg P/y)						
2001-2005	0.78	0.17	0.13 – 0.32	---	---	
2014-2018	0.74	0.19	0.13 – 0.37	---	---	-0.0003 – 0.051
Change	-0.03	0.02	-0.006 – 0.04			
% Change	-4	13	-4 – 13			

[a]Includes the Roanoke, Chowan, and Meherrin Rivers. [b]Includes Potecasi Creek, Cashie River, Scuppernong River, Little River, Perquimans River, and Kendrick Creek. [c]Range of potential load reflects an assumption of nutrient yields for the unassessed watershed area equal to the nutrient yields for the watersheds of the major rivers (lower bound estimate) or smaller assessed tributaries (upper bound estimate).

Table 4. Estimates of hardwood forest area harvested annually in the counties surrounding the Albemarle Sound system from 2002 to 2012.

County	Roundwood output (10^3 ft^3/y)	Total forest harvested[a] (ha/y)	Hardwood forest harvested[b] (ha/y)
North Carolina			
Bertie	22139	2684	886
Camden	2757	334	110
Chowan	4480	543	179
Currituck	1761	213	70
Dare	123	15	5
Gates	10783	1307	431
Halifax	17526	2124	701
Hertford	13268	1608	531
Hyde	3302	400	132
Pasquotank	4298	521	172
Perquimmans	6725	815	269
Tyrell	3699	448	148
Washington	5952	721	238
Virginia			
Chesapeake	1374	167	55
Southampton	21482	2604	859
Suffolk	5362	650	214
Virginia Beach	243	29	10
Total	**114491**	**13877**	**4580**

[a]Assumes a wood volume yield of 8250 ft^3/ha. [b]Assumes that the annual harvest of hardwood forest is 1/3 of total forest harvest.

Table 5. Potential impacts of swamp forest harvesting on increased nutrient loading to the Albemarle Sound system. Load increases to Albemarle Sound assume an annual harvest of 4600 swamp forest hectares.

Reference	Study description	Study TN yield increase (kg N/ha/y)	Potential Albemarle S. TN load increase (10^6 kg N/y)	Study TP yield increase (kg P/ha/y)	Potential Albemarle S. TP load increase (10^6 kg P/y)
Lebo and Herrmann 1998	Comparison of yields before and after clear cut harvest in pine silviculture	2.1-2.2	0.0097-0.010	0.12-0.36	0.00055-0.0017
Grace 2004	Before and after clear cut harvest compared to control in a hardwood, organic soil, swamp forest	35	0.16	0.12	0.00055
Reikerk 1983	Before and after clear cut harvest with 2 levels of reforestation disturbance compared to a control for flatwoods, pine silviculture	2.6 – 5.3	0.012-0.024	-0.04 – 0.07	-0.00018-0.00032
Wynn et al. 2000	Comparison of before and after clear cut harvest versus clear cut harvest with BMP implementation, versus control in a Virginia coastal plain, loblolly pine forest	18 - 81	0.083-0.37	-0.06 - 11	-0.0003 – 0.051
Range		**2.1 - 81**	**0.0097 – 0.37**	**-0.06 - 11**	**-0.0003 – 0.051**

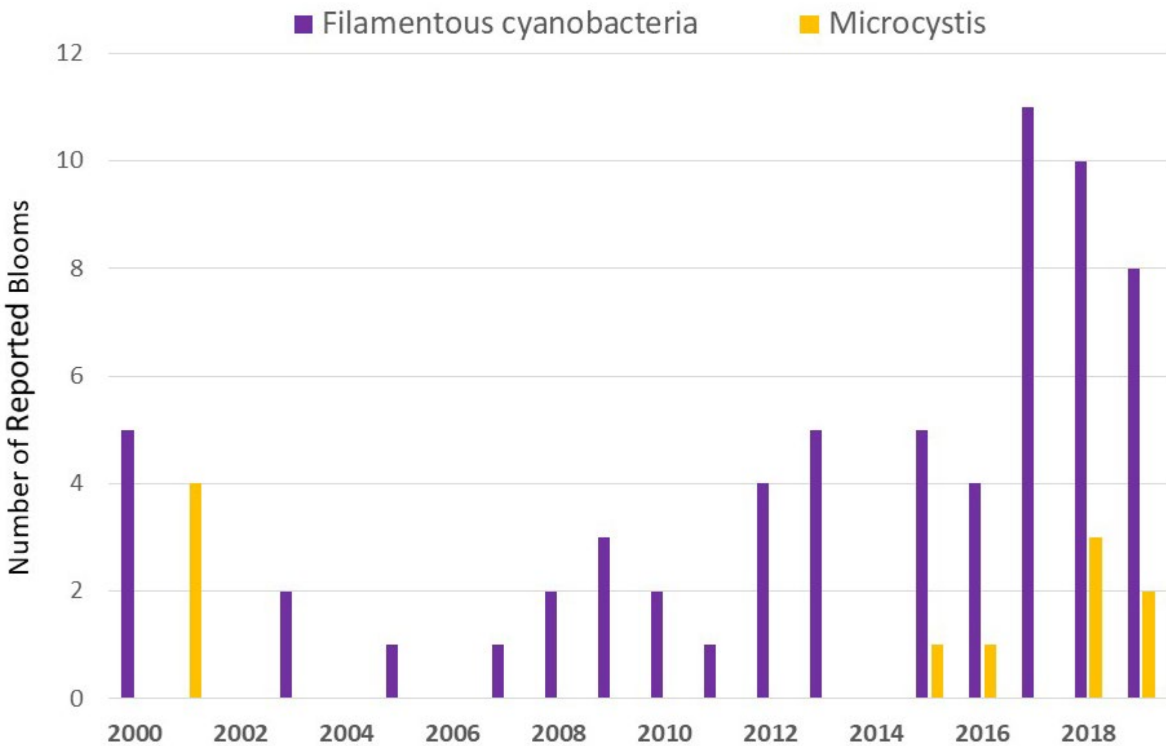

Figure 1. Number of algal blooms in the Albemarle Sound system reported to NC DEQ over the period 2000-2019. Data and figure provided by Elizabeth Fensin, NC DEQ-DWR.

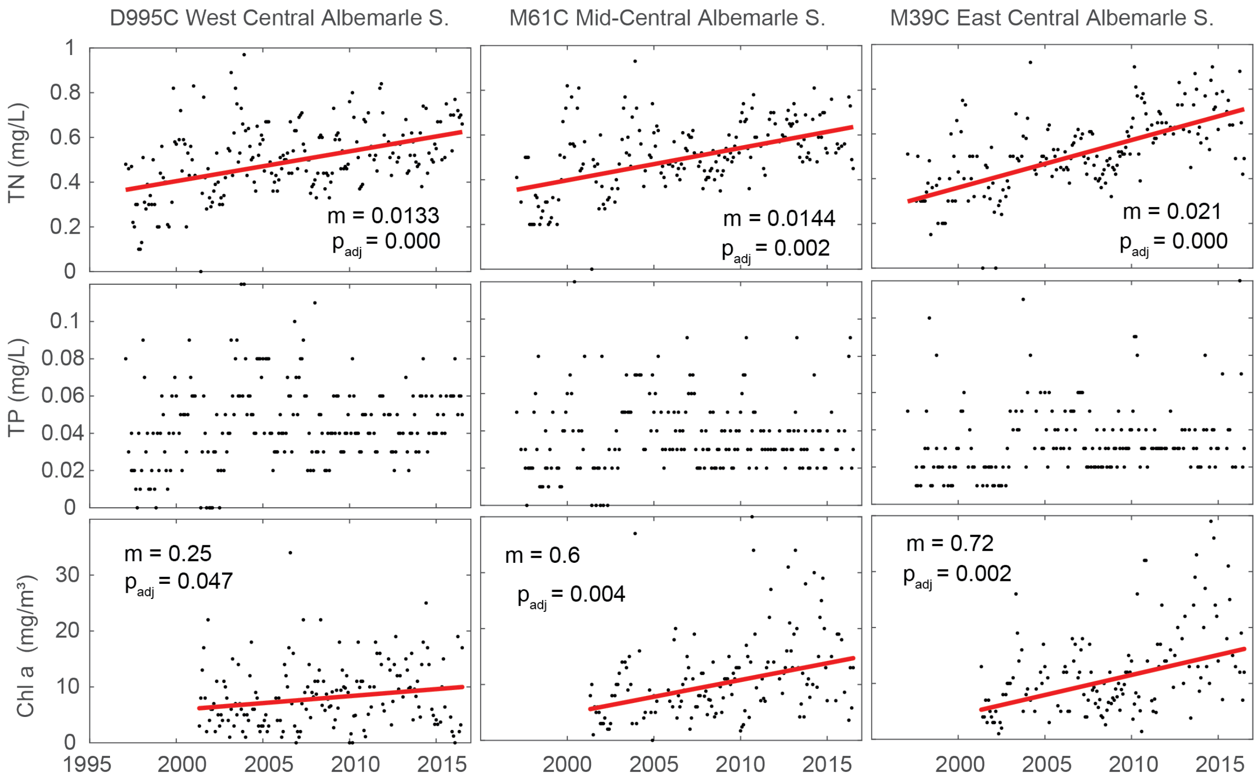

Figure 2. Time series of monthly total N and P, and chlorophyll a at stations along the main axis of Albemarle Sound. Solid red lines indicate the long-term trend when a statistically significant trend was detected by a seasonal Kendal test. P_{adj} values are adjusted p-values from the seasonal Kendal test, and m values are the Sen slope in the unit of measure per year.

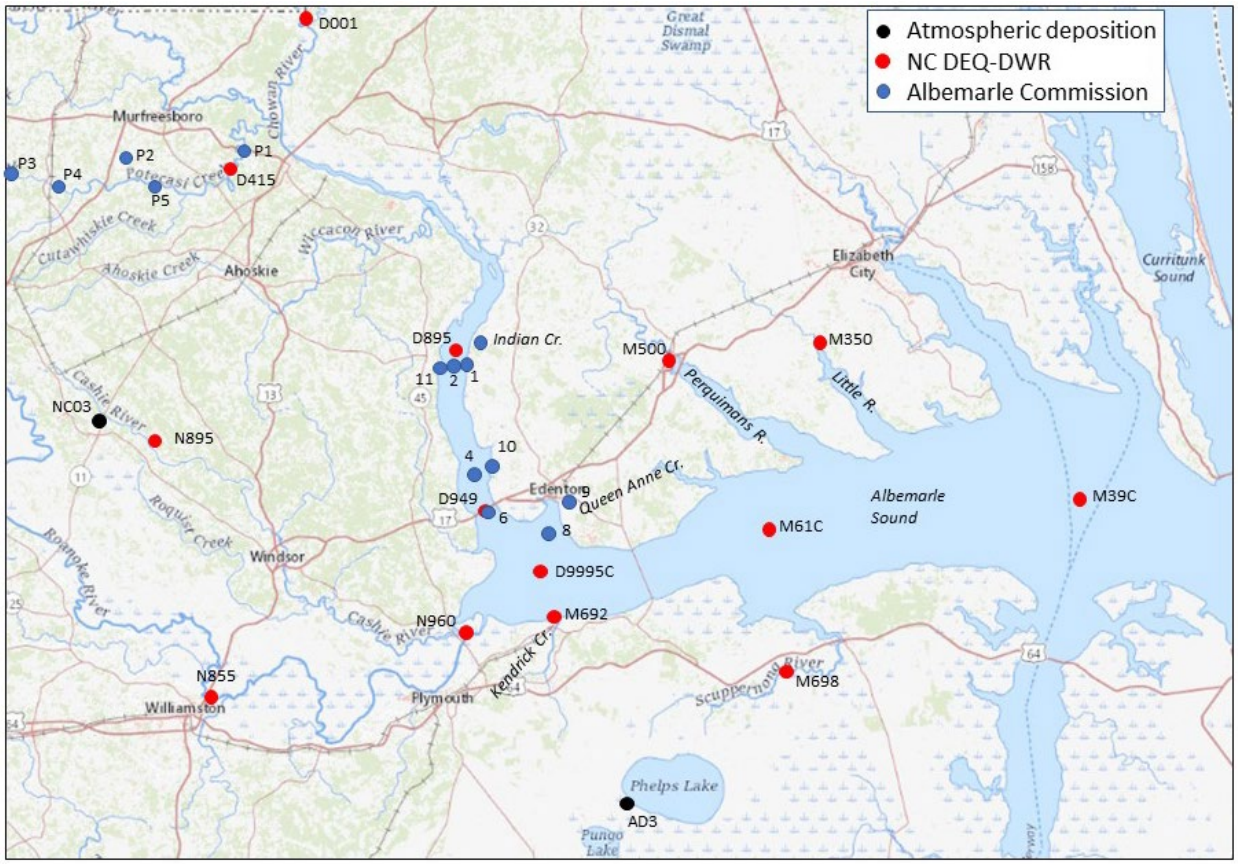

Figure 3. Map of the Albemarle Sound showing tributary streams and sampling locations for reported data.

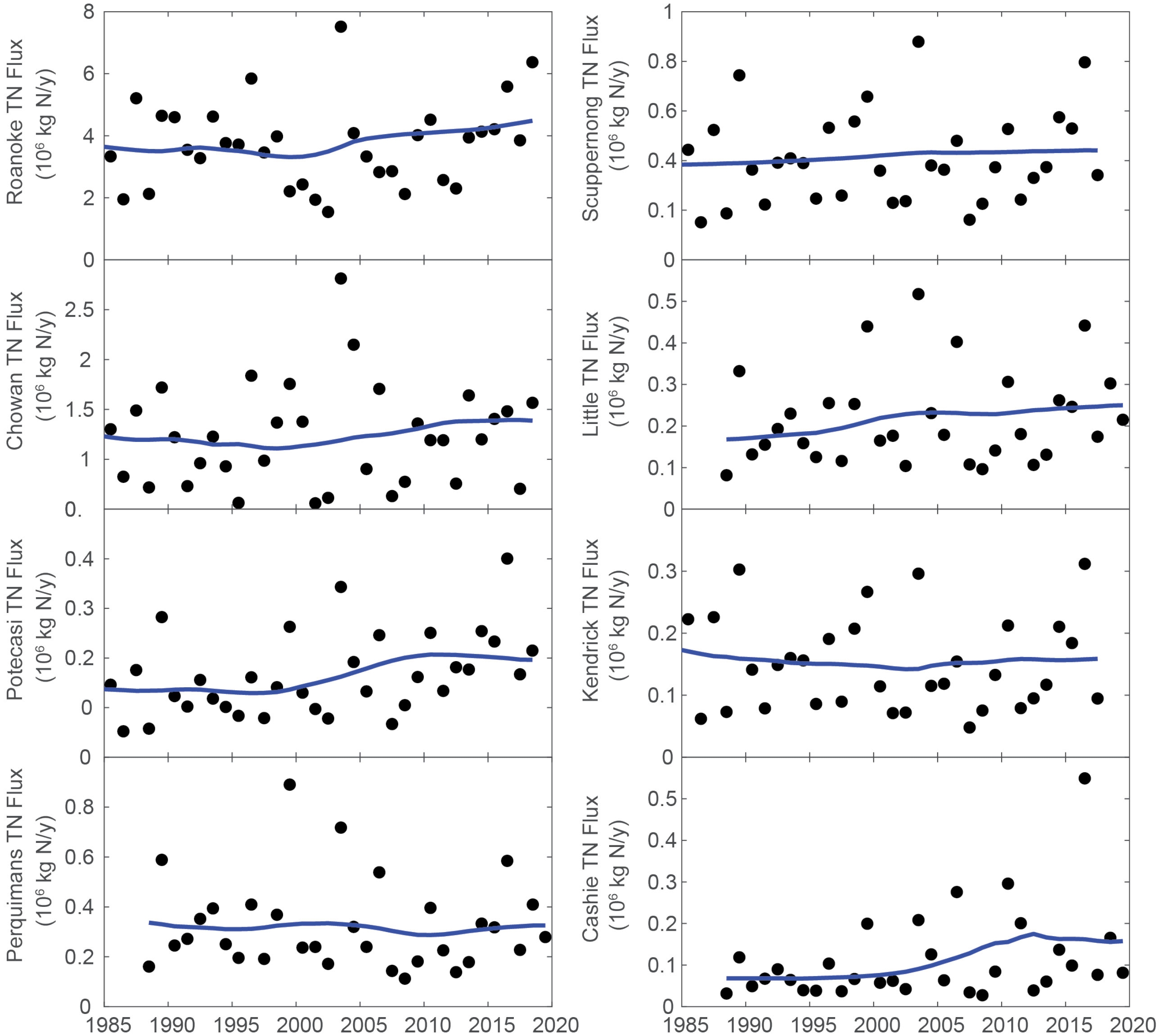

Figure 4. Time series of total N loads to the Albemarle Sound for six streams. Dots represent the annual load. Solid line represents the flow-normalized load.

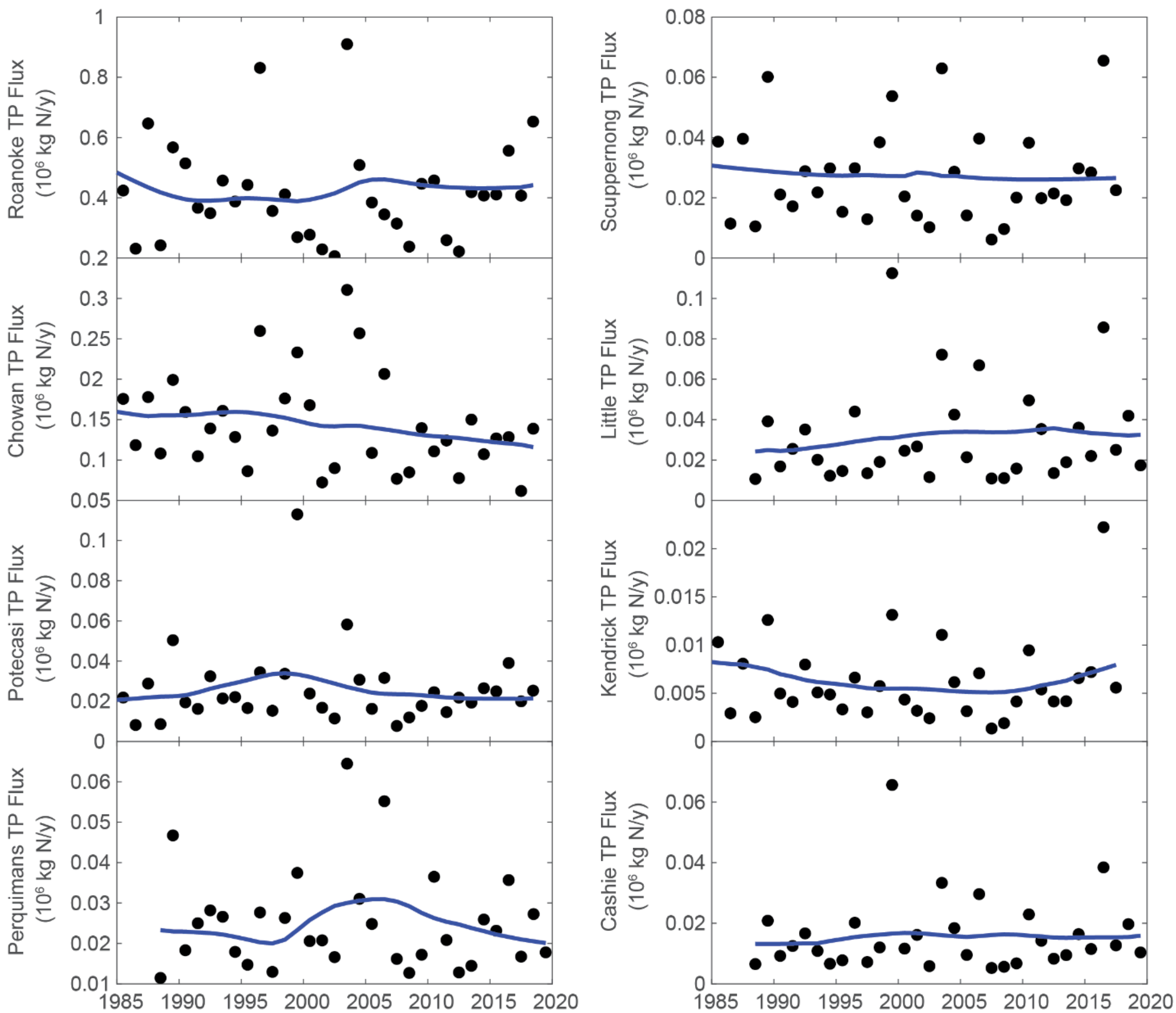

Figure 5. Time series of total P loads to the Albemarle Sound for six streams. Dots represent the annual load. Solid line represents the flow-normalized load.

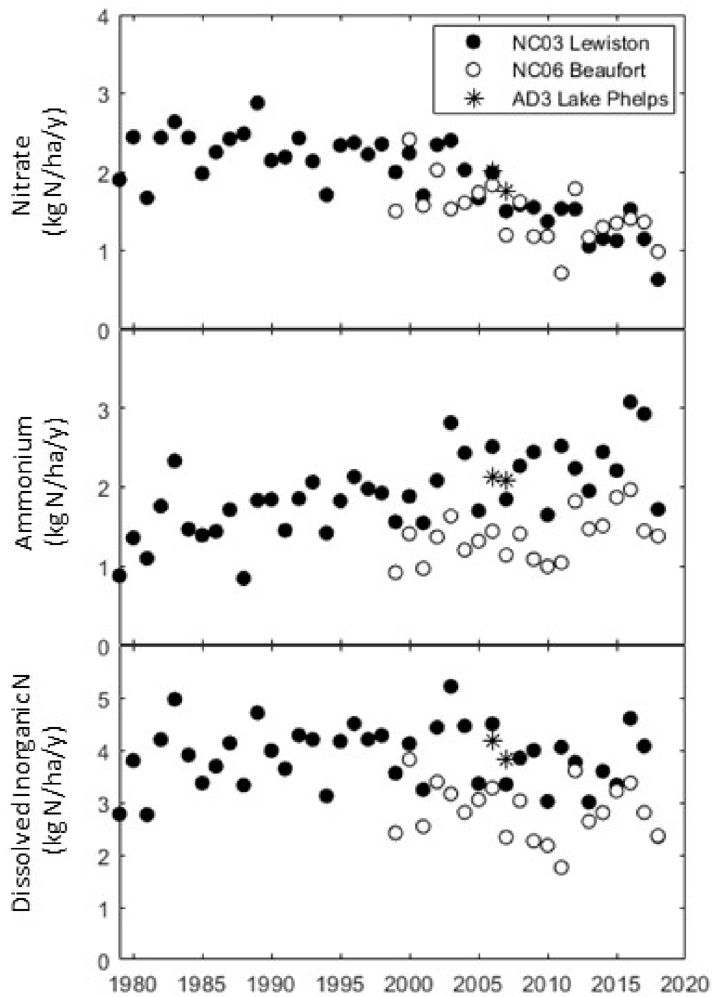

Figure 6. Time series of annual average atmospheric fluxes of nitrate, ammonium, and total dissolved inorganic nitrogen at National Atmospheric Deposition Program sites NC03 and NC06 near Lewiston and Beaufort, NC respectively, and site AD3 at Lake Phelps from the study by Rossignol et al. (2011).

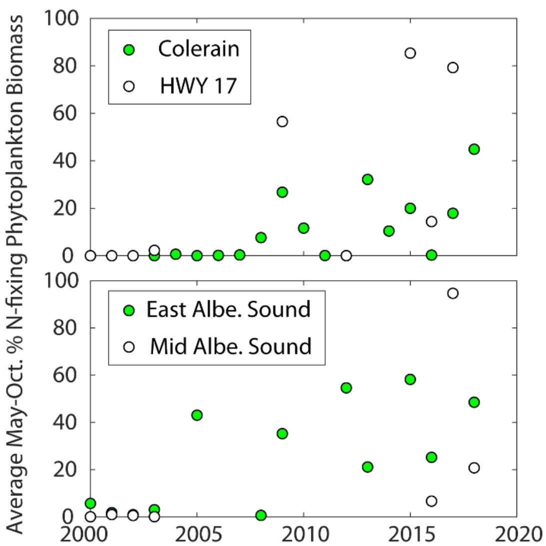

Figure 7. Time series of the annual average percentage of total phytoplankton biomass comprised by heterocystous cyanobacteria from May to October.

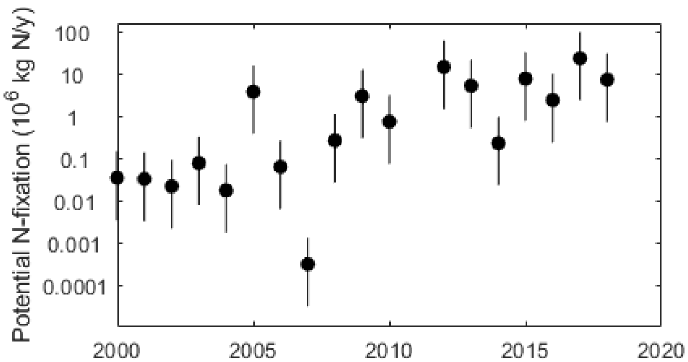

Figure 8. Time series of potential N fixation based on the annual average, microscopically determined, heterocystous cyanobacterial biomass at four stations in the Albemarle Sound system. Filled circles and errorbars represent the estimate based on the median, and 10th and 90th percentile values of N-specific, N fixation rates for natural heterocystous cyanobacteria communities (Klawonn et al. 2016).

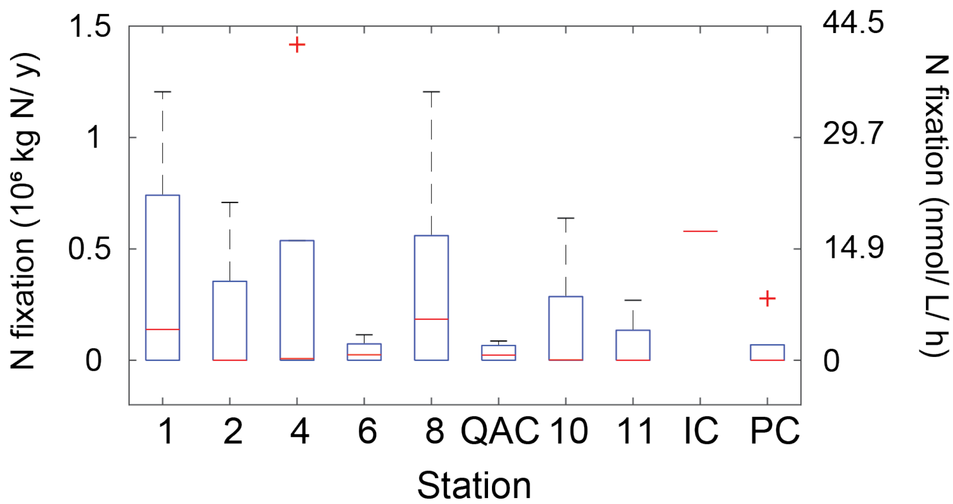

Figure 9. Box plots of measured N fixation rates at seven Chowan River stations and a location on each of Queen Annes Creek (QAC) and Indian Creek (IC), and five stations on Potecasi Creek (PC). Note all values on left y-axis have been scaled to a 12 h photoperiod, 180 d growing season and the volume of Albemarle Sound from the rate measurements on right y-axis. Red lines are the median. Boxes represent the interquartile range. Whiskers extend to 1.5 times the interquartile range, and + symbols identify outlying values beyond the whiskers.

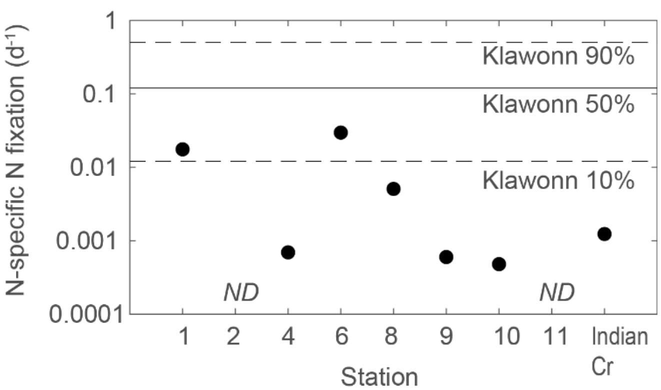

Figure 10. N-specific N fixation activity of heterocystous cyanobacteria in the Chowan River system on 30 August 2019. ND indicates not detected. In addition to no detectable N fixation, no heterocystous cyanobacteria were counted at station 11. Dashed and solid lines represent the 10 and 90% quantiles and median of rates of natural heterocystous cyanobacteria populations reviewed by Klawonn et al. (2016).