



Tracing nutrient pollution from industrialized animal production in a large coastal watershed

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Abstract One of the highest concentrations of swine and poultry concentrated animal feeding operations (CAFOs) in North America is located on the Coastal Plain of North Carolina, in which the Cape Fear River basin is located. The CAFOs produce vast amounts of manure causing loading of nutrients and other pollutants to receiving waters. With the Cape Fear River basin vulnerable to nutrient pollution, as are many other watersheds with CAFOs, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotopic signatures were identified from water samples collected within the Northeast Cape Fear, Black, and lower Cape Fear River watersheds to trace nutrient sources and their distribution downstream. The spatial and temporal variability of nutrients and isotopic signatures were also identified to understand water quality impacts of animal

waste spraying season and proximity to CAFOs. Our results showed that significantly enriched $\delta^{15}\text{N}$ signatures characterized sites in close proximity to CAFOs as well as point-source wastewater discharge areas, while the opposite was true for an unimpacted control stream and two estuarine sites. Additionally, the impacted sites yielded significantly ($p < 0.05$) higher nitrate concentrations than control and estuarine sites. Statistical analyses demonstrated that nitrate concentrations were positively correlated with heavier $\delta^{15}\text{N}$ signatures, suggesting that animal waste, as well as human wastewater, are relatively more important sources of N to this large watershed than fertilizers from traditional row crop agriculture. Our results also suggested that during appropriate hydrological conditions CAFO-derived N can be detected many kilometers downstream from freshwater sources areas to the estuary.

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Introduction

Industrial agriculture has become a norm for sustaining food production demands in recent decades and has been particularly congregated in certain rural communities in the USA. Industrialized livestock farms, recognized as concentrated animal feeding operations or CAFOs, have largely replaced the traditional agricultural practices of farrow-to-finish farms (McBride and Key 2013). Large corporations own swine, cattle, or poultry,

and contract animals out to individual landowners to raise hundreds to thousands of livestock and poultry in close confinement. This technique allows for mass production with lower costs. In the USA, North Carolina has the third highest industrial animal production concentration by state with 1222 registered CAFOs, only exceeded by Minnesota with 1300 and Iowa with 3588 (USEPA 2017). The environmental and water quality impacts of such industrialized production techniques have been documented in the literature (Burkholder et al. 2007; Mallin et al. 2015), but the impacts of such production on waterways farther downstream are less well-defined, and thus assessed in this study.

Swine and poultry CAFOs

The Cape Fear River watershed, the largest watershed in North Carolina, has the highest concentration of swine CAFOs in the world as well as numerous poultry CAFOs (Cahoon et al. 1999; Mallin and Cahoon 2003). The magnitude of livestock and poultry in this area increases the demand for large quantities of animal feed, mainly comprised of soybeans and cornmeal (Choi 2007). These primary feed grains are imported to Mid-Atlantic states from outside states that specialize in corn and soybean agriculture because facilities cannot grow enough of their own feed to meet the demand themselves, and feed importation is a low-cost option. CAFOs located within the Cape Fear River basin in North Carolina import over 90% of soybeans for animal feed (Cahoon et al. 1999). While low-cost feed imports have facilitated the growth of CAFOs in North Carolina, they have also increased external, “new,” nitrogen (N) and phosphorus (P) influxes to affected watersheds. With the uneven distribution of swine CAFOs across the USA, 13% concentrated in North Carolina, the Cape Fear River watershed is biogenically stressed with excess nutrient and fecal microbial influxes (Kellogg 2000; Harden 2015).

One of the leading environmental concerns brought about by the industrial agriculture system is the quantity and management of waste produced (USEPA 2004). The high-density animal confinement of CAFOs yield industrial levels of waste that have surpassed human waste production 13-fold (Burkholder et al. 2007). CAFO manure specifically threatens water quality degradation with nutrient contamination (USEPA 2004). Concentrated swine waste management and storage practices are generally characterized by the disposal

and storage of waste water, comprised of swine feces and urine, in outdoor lagoons which are seasonally pumped out and sprayed on surrounding fields, termed “spray fields” (Mallin et al. 2004; Mallin et al. 2015). Within the common swine CAFO, waste accumulates in each individual stall and throughout the containment facility. The floors are comprised of slats that have slits allowing for manure to be hosed down through and drop into an underground conveyance below for storage, prior to being flushed out into the waste lagoons (Mallin et al. 2015). Anaerobic lagoons act as a treatment of wastewaters, converting organic material into stable compounds such as carbon dioxide and methane that are volatilized (USEPA 2002). After the treatment period, in which dense organic sludge settles out and separates from supernatant liquid wastewater, the supernatant is sprayed on nearby fields containing a cover crop, usually Bermudagrass; spraying is permissible March through September (Mallin and McIver 2018). The nutrients from the concentrated waste are assumed to be absorbed by the cover crops on the swine waste spray fields. In traditional, farrow-to-finish farms, this method was viable because manageable waste amounts could be upcycled into fertilizer for their own swine forage (Hribar 2010). However, when this common practice is transitioned to CAFOs, the manure quantity exceeds land and/or soil nutrient capacity (Haines and Staley 2004).

Poultry CAFOs are also abundant in North Carolina, though the exact number remains unknown as dry litter poultry operations are not required to obtain permits. There are currently 19 wet-litter poultry CAFOs with permits in North Carolina (egg-laying facilities) while the vast majority of poultry CAFOs utilize dry litter waste disposal. Dry litter poultry farms are defined in that manner as the poultry waste (i.e., litter) is dried and spread on surrounding fields or shipped to other sites to be applied as nutrient fertilizer (Harden 2015). Overall, environmental contamination from swine and poultry CAFOs results from oversaturated spray field soils leaching into ground water during routine spraying, runoff from over capacitated lagoons or freshly sprayed fields during precipitation events and hurricanes, or leakage from poor lagoon construction (Amini et al. 2017; Burkholder et al. 2007). The magnitude of environmental impacts that CAFO wastewater imposes depends on the contaminants involved, soil properties, and proximity to waterways (Huddleston 1996; Burkholder et al. 2007). Close proximity of numerous CAFOs to

streams and floodplains, apparent in North Carolina, facilitates vulnerability to wastewater runoff contaminants during storms and lagoon overflow (Wing et al. 2002; Martin et al. 2018).

Cape Fear River watershed

The Southeastern region of North Carolina, in which the Cape Fear River watershed is located, is physiographically characterized as Coastal Plain (NCDC 2018). The Coastal Plain is comprised of two main sub-areas, tidewater and interior portion (NCDC 2018). Tidewater areas, closest to the ocean, are characteristically flat, swampy, and poorly drained. The interior region has a gentle slope with good drainage. Overall, the Coastal Plain soil composition is soft sediment with generally no hard rock substrate beneath the surface (NCDC 2018). Wetlands dominate the Coastal Plain, particularly the tidewater areas, and have demonstrated water quality benefits through sediment and nutrient sequestration (Robinson 2005). The central region of North Carolina is described as Piedmont, located between the Coastal Plain and Mountain regions. The Cape Fear River watershed originates within the Piedmont and flows southeast crossing the fall line into the Coastal Plain and eventually emptying into the Atlantic Ocean. The lower 40 km of the river comprises the Cape Fear Estuary.

Watersheds with swine CAFOs have been found to contain streams with considerably higher inorganic and organic N concentrations than unimpacted streams (Harden 2015; Mallin et al. 2006, 2015). Anaerobic waste lagoons with large quantities of organic N produce high concentrations of ammonium-N through deamination (Mallin and Cahoon 2003), some of which is nitrified following discharge into the environment. The ability for nitrate to leech into groundwater depends on underlying soil composition and groundwater depth. High permeability soil (i.e., gravel, sandy gravel, and coarse sand) and relatively low aquifer depth are associated with high nitrate concentrations in groundwater (USEPA 2004). The North Carolina Coastal Plain generally has porous soils and a high water table, around 0–2 m below the surface, which create vulnerability to nutrient leeching (Mallin et al. 2015; USGS 2019). Thus, underlying geological and hydrological conditions exacerbate off-site transport of nutrients to downstream water bodies. Tracing of off-site nutrient loading can be accomplished through stable isotope studies.

The Cape Fear River region and surrounding coastal areas have been ranked most vulnerable to manure nutrient pollution within the USA (Kellogg 2000). Over 50% of North Carolina's swine production is located in the Cape Fear River Basin, where lagoon effluent is able to enter the blackwater streams that dominate such watersheds (Mallin et al. 2015; Martin et al. 2018). Unimpacted blackwater systems are characteristically inorganically nutrient poor and have low dissolved oxygen levels in comparison to anthropogenically induced streams (Smock and Gilinsky 1992). Phytoplankton production in these systems is stimulated by N, rather than P; however, P inputs directly stimulate bacterial growth; thus these systems are susceptible to biochemical oxygen demand (BOD)-induced oxygen depletion from loading of either nutrient (Mallin et al. 2004, 2006). CAFO-dominated watersheds of blackwater streams have the potential to drain substantial nutrient loads into the two major 5th-order tributaries (Northeast Cape Fear River and Black River) of the Cape Fear River, which subsequently enters the Atlantic Ocean through its estuary (Fig. 1). It is essential to assess nutrient concentrations and sources within the watershed to understand and mitigate nutrient pollution to the larger rivers and estuary.

Particulate organic matter ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$)

Particulate organic matter (POM) is characterized as particles that range from 0.054 to 2.0 mm, and physically cannot pass through a 7- μm filter pore (Cambardella and Elliott 1992; Nebbioso and Piccolo 2013). Organic carbon (C) and nitrogen (N) stable isotope ratios have been identified as natural tracers for organic matter origins and seasonal influxes (Thornton and McManus 1994). These isotopic ratios are assumed to reflect a distinct end-member that correlates with the biogeochemical process that alters their composition. Organic matter pools within river sediments are a conglomerate of biogenic processes, and isotopic mixing models estimate contributions from the assortment of various organic sources.

As mentioned previously, the application and storage of manure is associated with ammonia volatilization. When ammonia volatilizes during wastewater degradation, the lighter $\delta^{14}\text{N}$ is removed leaving a majority of heavier $\delta^{15}\text{N}$ isotopes in the soil or waste ponds (Macko and Ostrom 1994). Bacterial nitrification in soil produces nitrate from ammonium, still rich in $\delta^{15}\text{N}$. POM $\delta^{15}\text{N}$ signatures have been distinguished for various nitrogen

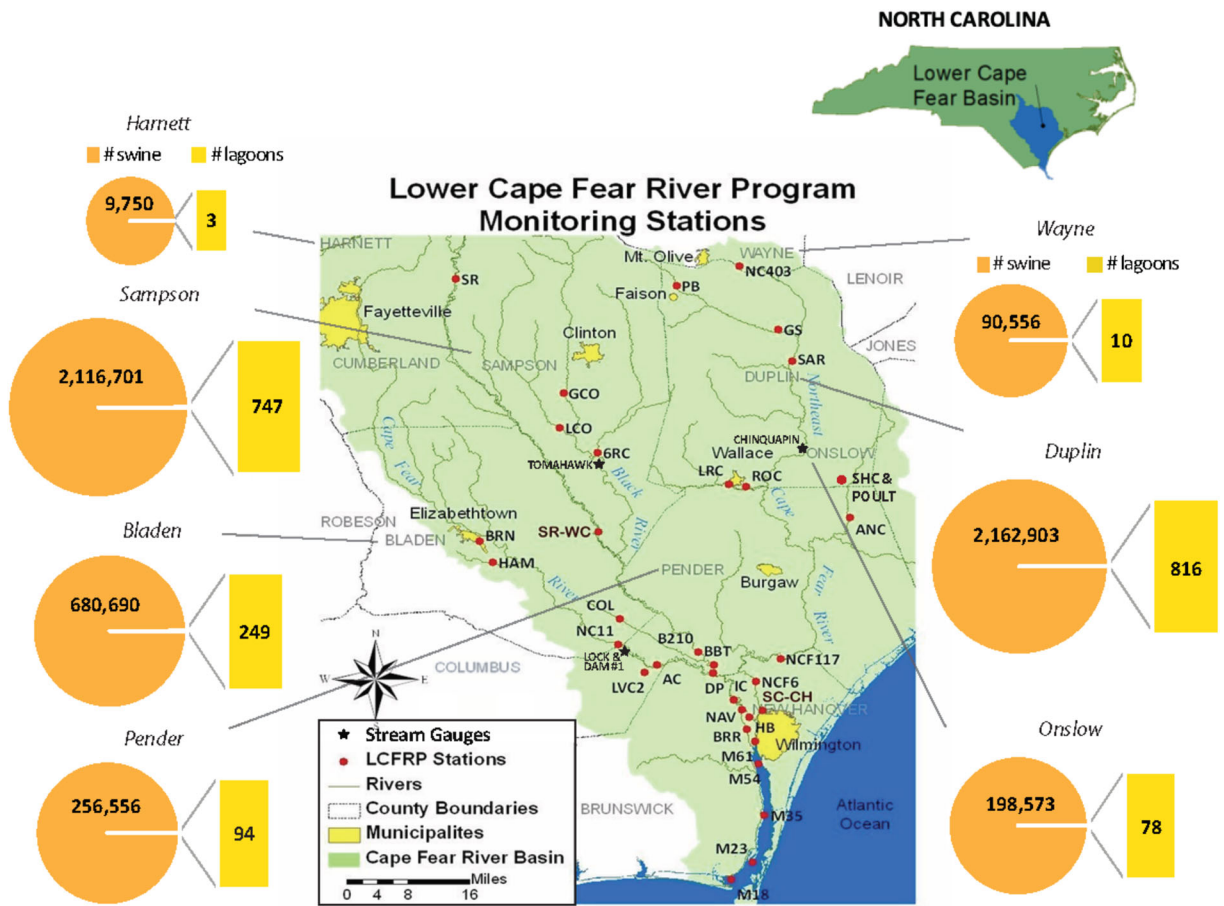


Fig. 1 Map of the Lower Cape Fear River Program monitoring stations showing sampling locations for this study. Inset shows location of sampling area within North Carolina, USA. Additional

information includes standing crop of swine and number of waste lagoons per county (NCDEQ 2016)

source inputs (Table 1). Fixation of atmospheric N produces $\delta^{15}\text{N}$ signatures near zero (Kendall and McDonnell 1998), and atmospheric deposition of N produced signatures around -3.0‰ (Heaton 1986). Synthetic fertilizers are generally in the -2.0 to $+2.0\text{‰}$ range (Bateman and Kelly 2007). Signatures of both human and animal waste are generally in the $+10.0$ to $>+20.0\text{‰}$ range, depending on degree of treatment or amount of microbial conditioning in the soil (Heaton 1986; Costanzo et al. 2001). The poultry waste signature generally ranges between $+8.0$ and $+16.0\text{‰}$, centered around $+8.0$ to $+9.0$ (Wassenaar 1995); although poultry manure pellets have displayed $\delta^{15}\text{N}$ signature as low as $+5.0\text{‰}$ (Bateman and Kelly 2007). Thus, for human and animal waste, there is a considerable overlap, and distinguishing specific sources of N pollution can be aided by knowledge of the watershed land use and pollution sources (Lapointe et al. 2017). With deposition

from atmospheric nitrogen, groundwater $\delta^{15}\text{N}$ typically ranges from $+2$ to $+8\text{‰}$ (Macko and Ostrom 1994). Marine aquatic sources have a broader range of $\delta^{15}\text{N}$, from -15 to $+20\text{‰}$ (Fernandes et al. 2016).

In addition to $\delta^{15}\text{N}$, the $\delta^{13}\text{C}$ signatures identify plant characteristics of specific water types (i.e., freshwater, estuarine, or seawater) to better understand the origin of carbon throughout the watershed. Freshwater plankton typically exhibit $\delta^{13}\text{C}$ signatures from -30 to -25‰ , while C3 and C4 estuarine plants have heavier $\delta^{13}\text{C}$ signatures ranged -27‰ to -13‰ (Fry 2006). Carbon source nutrients from livestock and poultry waste can be identified through $\delta^{13}\text{C}$ signatures that reflect the plant material they eat, generally within the range of -27‰ to -22‰ (North et al. 2004). Overall, isotope tracing holds the potential to determine sources of environmental impact related to CAFO pollution distributed throughout a watershed.

Table 1 Overview of $\delta^{15}\text{N}$ signatures from various sources (from Heaton 1986; Wassenaar 1995; Costanzo et al. 211; Bateman and Kelly 2007; Lapointe et al. 2017)

Source	Range
Natural N-fixation	0‰
Atmospheric N	- 3‰ to + 1‰
Synthetic fertilizer	- 2‰ to + 2‰
Human wastewater (depends on degree of treatment)	+ 3‰ to + 19‰
Livestock waste	+ 10‰ to > + 20 ‰
Poultry waste (depends on poultry diet)	+ 5‰ to + 16 ‰

The CAFO-rich Cape Fear River watershed is vulnerable to the spread of nutrient pollution throughout the system (Mallin et al. 2004, 2006), and thus the primary objective of this study was to track and assess the presence of swine and poultry CAFO nutrient pollutants in the Cape Fear River watershed downstream through the Cape Fear River Estuary using $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic tracers. Additionally, the wide distribution of CAFOs throughout the watershed and periodic spraying of manure suggests the presence of seasonal and geographic distributions in water quality. Thus, the secondary objective was to assess spatial and temporal variability of nutrients (nitrogen and phosphorus), and isotopic signatures of POM in various streams and tributaries within the Cape Fear River watershed in relation to CAFO influence.

Materials and methods

Study sites

The lower Cape Fear River Basin is comprised of a series of rivers, streams, and tributaries that are subject to water quality fluctuations resulting from traditional agriculture, the numerous CAFOs present in the basin, and human and industrial point-source waste discharges permitted by the National Pollution Discharge Elimination system (NPDES). Ten sites were selected for sampling based on CAFO or point-source influence or lack thereof (Table 2). These sites are a subset of a greater and ongoing (since 1995) monitoring effort called the Lower Cape Fear River Program (Fig. 1). Two fifth-order blackwater streams, the Black River, and the Northeast Cape Fear River, together house approximately 5,000,000 head of swine and numerous poultry

operations and flow downstream to connect with the main Cape Fear River upstream of the port city, Wilmington, NC (Fig. 1). The Black River is fed by tributaries, such as Six Runs Creek (6RC) that mainly drains watersheds used for CAFOs and agriculture, and Colly Creek (COL) which drains primarily undeveloped forest area and is considered a control site (Mallin et al. 2004, 2006). Station NC403 is a site in the headwaters of the Northeast Cape Fear River and receives influence from one NPDES point-source discharge (total 1.4 MGD), several CAFOs, and a cattle farm. Panther Branch (PB) and Stockinghead Creek (SHC) are tributaries of the Northeast Cape Fear River. PB is primarily impacted by an NPDES wastewater treatment plant outfall (0.5 MGD). The SHC watershed contains approximately 13 swine and 11 poultry CAFOs (Mallin and McIver 2018). A large poultry CAFO (POULT) was sampled in a perennial ditch that drains the facility and enters public waters. The main Cape Fear River watershed includes two tributaries selected for this study. One is the Browns Creek (BRN) watershed which contains three swine CAFOs but also encompasses 3500 resident Elizabethtown. The other is Hammond Creek (HAM) watershed which contains four poultry CAFOs and 13 swine CAFOs, including one very large CAFO (2500 or more swine heads). The watersheds of both creeks also are used for crop agricultural purposes (tobacco, sweet potatoes, corn, hay). The mainstem of the Cape Fear River flows downstream through Wilmington, and eventually discharges into the Atlantic Ocean in Southport, NC. Channel Marker 61 (M61) in downtown Wilmington and Channel Marker 18 (M18) in Southport, NC were sampled to investigate the influence of upstream watershed drainage to the Atlantic Ocean.

Sample collection

Of the ten sites within the Cape Fear watershed (Fig. 1, Table 2), eight were sampled monthly from February to August 2018; all sampling was suspended in September due to the approach of Hurricane Florence. Two sites, SHC and POULT were sampled in March (to represent low sprayfield influence) and in June (2 months after the onset of permissible swine waste spraying). At each site, a YSI Professional Plus unit with Quatro Multiparameter probe was used to measure temperature, salinity, dissolved oxygen (DO), turbidity, pH, and specific conductance (not presented in this paper). Water was collected at the surface using a bucket and rope, as the sites

Table 2 Site locations for sampling including coordinates and description (see also Fig. 1; EWG 2016; Waterkeeper Alliance; site locations on Lower Cape Fear River Program website <https://uncw.edu/cms/aelab/lcfrp/>)

Site	Coordinates	Description
COL	34.4641, - 78.2569	Colly Creek, 2nd order, control site located in a largely pristine blackwater wetland area; a tributary of the Black River; four swine CAFOs
NC403	35.1784, - 77.9807	Northeast Cape Fear River headwaters, 1st order, drains a watershed that hosts nine swine CAFOs, traditional agriculture; grazing cattle; an NPDES point-source waste water discharge (1 MGD)
PB	35.1345, - 78.1363	Panther Branch, 1st-order tributary of Northeast Cape Fear River, receives a NPDES point-source waste water discharge (0.5 MGD); one poultry CAFO in watershed
6RC	34.7933, - 78.3113	Six Runs Creek, 3rd-order tributary of the Black River, high influence of CAFOs (179 swine and 107 poultry CAFOs)
BRN	34.6136, - 78.5848	Browns Creek, 2nd-order tributary of the Cape Fear River, presence of three swine CAFOs and traditional agriculture; drains Elizabethtown (3500 residents)
HAM	34.5685, - 78.5155	Hammond Creek, 2nd-order tributary of the Cape Fear River; 13 swine CAFOs and four poultry CAFOs, traditional agriculture
M61	34.1938, - 77.9573	6th order, Cape Fear River at the State Port downtown Wilmington- estuarine system that receives water from Cape Fear River and Northeast Cape Fear River confluence
M18	33.913, - 78.017	6th order, Cape Fear River at South Port, lower end of the estuary that opens into the Atlantic Ocean
SHC	34.50305, - 77.51554	Stocking Head Creek, 2nd-order stream, site located along a swine CAFO spray field; 13 swine and 11 poultry CAFOs
POULT	34.50308, - 77.51548	1st-order, perennial drainage ditch with direct poultry CAFO influence; one large poultry CAFO

are located under bridges and are approximately 1–2 m in depth. Water samples were collected in duplicate 2 L, acid-washed (10% HCl) Nalgene bottles. Sample water was filtered on site through baked (550 °C for 4 h) 24-mm Whatman GF/F glass fiber filters (0.7 mm pore size) using a syringe and collected in acid-washed test tubes and baked clear glass vials. Once collected, all tubes and vials were placed on ice and transported back to the lab where they were frozen at -20 °C until analyses for nutrients and dissolved organic matter.

Upon return to the lab, water samples in the 2-L Nalgene bottles were filtered through baked 47-mm Whatman GF/F filters. At least 500 mL of sample water from each bottle were filtered through an individual filter, and duplicates were produced for each site. The volume of water pumped through each filter was recorded to the nearest 0.1 mL. Once the filter was sufficiently full of organic material that water could no longer pass through, the filter was removed from the tower, wrapped in foil and frozen (-80 °C) until analyses for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures.

Nutrient analysis

All water samples that were filtered were analyzed for dissolved inorganic and organic nutrients. Dissolved

inorganic nitrogen (DIN), as nitrite NO_2^- , nitrate NO_3^- , and ammonium NH_4^+ , and total dissolved nitrogen (TDN), as well as dissolved inorganic phosphorus (DIP as orthophosphate, PO_4^{3-}), and total dissolved phosphorus (TDP) were analyzed using the Bran+Luebbe AutoAnalyzer 3. When nitrate is referenced henceforth, it will refer to the concentration of nitrate plus nitrite. TDN was determined using Koroleff's wet alkaline persulfate oxidation analysis where water samples were prepared with recrystallized persulfate solution and autoclaved. Samples with higher organic content were diluted 5:1 or 6:1 deionized water to sample ratios prior to analysis. The samples were digested to nitrate (NO_3^-) and phosphate during analysis of TDN and TDP. The following equations were used to determine the concentration of dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) from TDN, DIN, TDP, and DIP (Koroleff 1983):

$$[\text{DON}] = [\text{TDN}] - [\text{DIN}]$$

$$[\text{DOP}] = [\text{TDP}] - [\text{DIP}]$$

A variety of ancillary physical, chemical, and biological samples are collected monthly at most of the sampled sites and the state-certified data following QA/QC

procedures are published on the Lower Cape Fear River Program website: <https://uncw.edu/cms/aelab/lcfrp>.

POM $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic analysis

For $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic analyses, POM filter samples were removed from the freezer and 15 mm diameter cores were punched from each filter. The glass fiber filter cores were weighed, then dried at 60 °C for 3–5 h and weighed again. Samples were then fumed in a desiccator with concentrated HCl to remove remaining inorganic carbonates present. Once dried again, samples were packed into tin capsules and placed into a sample tray for analysis on the ThermoDelta V Plus/Costech 4010 Elemental Analyzer to determine isotopic signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The isotopic signatures of carbon and nitrogen were recorded as del notations and calculated using the isotope of the samples and standards as shown below:

$$\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

Vienna Pee Dee Belemnite was used as the stable carbon isotope standard, and atmospheric nitrogen (N_2) was the measure for the stable nitrogen isotope standard.

Statistical analyses

All data collected were recorded, archived, and organized using Microsoft Excel 2008. Statistical analyses were conducted using SAS statistical software (Schlotzhauer 2009). Nutrient, isotopic and hydrological data were first tested for normality using the Shapiro-Wilk test, with nitrate, ammonium, river discharge, and rainfall requiring log-transformation to achieve normality. Correlation analysis was used to test for associations among chemical and hydrological variables. Analysis of variance (ANOVA) was used to test for differences in mean nutrient concentrations and isotopic signatures among sites. Where significant differences occurred, sites were ranked using the least significant difference (LSD) procedure (Day and Quinn 1989). Statistical tests were considered significant if $P < 0.05$.

River discharge was used as a proxy for streamflow, since most individual streams in southeastern NC are not gauged. Data were used from USGS sites 02105769 (mainstream Cape Fear River at Lock and Dam #1), 02106500 (Black River near Tomahawk, NC), and

02108000 (Northeast Cape Fear River near Chinquapin, NC). The average discharge of the 7 days prior to sampling was used for statistical purposes, with data from Lock and Dam #1 used for BRN, HAM, M61, and M18; Tomahawk data were used for COL and 6RC, and Chinquapin data were used for NC403 and PB. Rainfall data for the day of sampling plus the preceding 48 h was used for statistical purposes, with the nearest official NC rain gauges (either from USGS or the NC State Climate Office) to the site in question used as sources.

Results

Dissolved nitrogen concentrations

Nitrate ($\text{NO}_3^- + \text{NO}_2^-$), exhibited the highest mean concentrations at station NC403 (68.8 $\mu\text{mol/L}$), followed by station 6RC (47.6 $\mu\text{mol/L}$) (Figs. 2a and 3d, f). The peak individual nitrate concentration occurred in March at NC403 with 124.8 $\mu\text{mol/L}$ and the lowest nitrate concentrations were found from March to August at the unimpacted site COL (Figs. 2a and 3a). Nitrate concentrations at NC403 were significantly ($p < 0.05$) higher than all other sites except for 6RC, which in turn was significantly higher than all sites except PB and NC403. COL also had the lowest ($p < 0.05$) average concentrations of nitrate (2.24 $\mu\text{mol/L}$) among sites except for M18, and ammonium levels frequently exceeded that of nitrate more than any other station. March and July at M18 (nearest the ocean) had the lowest concentrations of ammonium (0.99 and 1.58 $\mu\text{mol/L}$), followed by June and August at 6RC (2.10 and 2.04 $\mu\text{mol/L}$). The highest values of ammonium occurred at PB in May (35.4 $\mu\text{mol/L}$) and HAM in February at 19.24 $\mu\text{mol/L}$. Average ammonium at PB was greater ($p < 0.05$) than 6RC, BRN, M61, and M18, with NC403 > 6RC and M18. Thus, highest inorganic N concentrations occurred at PB, N403, and 6RC. There was a general trend of higher nitrate concentrations during the start of spring (February, March, and April). COL exhibited the highest mean DON concentrations ($p < 0.05$), yet station 6RC had the highest individual DON overall in April (Figs. 2b and 3a, d).

At SHC, nitrate levels increased from March to June with a concentration of 18.6 to 26.5 $\mu\text{mol L}^{-1}$ (Fig. 4a). Ammonium concentrations nearly doubled from March to June at SHC with concentrations of 1.8 to

3.2 $\mu\text{mol L}^{-1}$ (Fig. 4a). DON concentrations for SHC also increased from March to June at 43.9 to 51.3 $\mu\text{mol L}^{-1}$ (Fig. 4a).

At POULT, nitrate concentrations were only 1.05 $\mu\text{mol L}^{-1}$ in March but increased to 28.8 $\mu\text{mol L}^{-1}$ in June (Fig. 4b). Ammonium concentrations at POULT were temporally similar to SHC with 2.04 $\mu\text{mol L}^{-1}$ in March and 3.43 $\mu\text{mol L}^{-1}$ in June (Fig. 4b). There was a small increase in DON concentrations from March with 55.5 $\mu\text{mol L}^{-1}$ to June with 60.7 $\mu\text{mol L}^{-1}$ at POULT (Fig. 4b). Overall, both SHC and POULT showed a considerable increase in nitrate and DON concentrations and a relative increase in ammonium concentrations from March to June. All other stations, except M18, had a general decrease in nitrogen concentrations from March to June.

Dissolved phosphorus concentrations

DIP concentrations ranged from undetectable to 1.87 $\mu\text{mol L}^{-1}$ (Fig. 5a–h). DOP concentrations ranged from 0.26 $\mu\text{mol L}^{-1}$ up to 2.66 $\mu\text{mol L}^{-1}$ (Fig. 5a–h). DIP concentrations were overall highest at COL with the maximum at 2.66 $\mu\text{mol L}^{-1}$, while DOP concentrations were lowest at COL with only one detectable data point at 0.20 $\mu\text{mol L}^{-1}$ (Fig. 5a). Generally, lowest DIP and DOP concentrations occurred in April and May, and beginning in June there was an increase in both DIP and DOP concentrations that remained higher through August at each station (Fig. 5a–h). PB, BRN, HAM, and 6RC showed the highest concentrations of DP (Fig. 5b–e). The estuarine stations M61 and M18 had the lowest DOP concentrations ranging from 0.00 to 0.85 $\mu\text{mol L}^{-1}$ (Fig. 5g, h). Lower DIP concentrations were found at stations PB and BRN, with averages of 1.12 and 0.86 $\mu\text{mol L}^{-1}$. Overall, M18 had the lowest average DIP and DOP, and COL had nearly zero DOP concentrations, yet the highest average DIP. Average DIP concentrations at both COL and NC403 were significantly ($p < 0.05$) higher than DIP at PB, BRN, and M18. Overall, none of the DIP samples was particularly high.

DIP concentrations at SHC doubled from March with 1.05 $\mu\text{mol L}^{-1}$ to June at 2.5 $\mu\text{mol L}^{-1}$ (Fig. 4c). DOP increased substantially from 0.14 $\mu\text{mol L}^{-1}$ in March to 1.49 $\mu\text{mol L}^{-1}$ in June at SHC (Fig. 4c). At POULT, the DIP concentration increased 15 \times from 0.27 $\mu\text{mol L}^{-1}$ in March to 4.1 $\mu\text{mol L}^{-1}$ in June (Fig. 4d). DOP concentrations at POULT increased temporally from

0.40 $\mu\text{mol L}^{-1}$ in March to 1.67 $\mu\text{mol L}^{-1}$ in June (Fig. 4d).

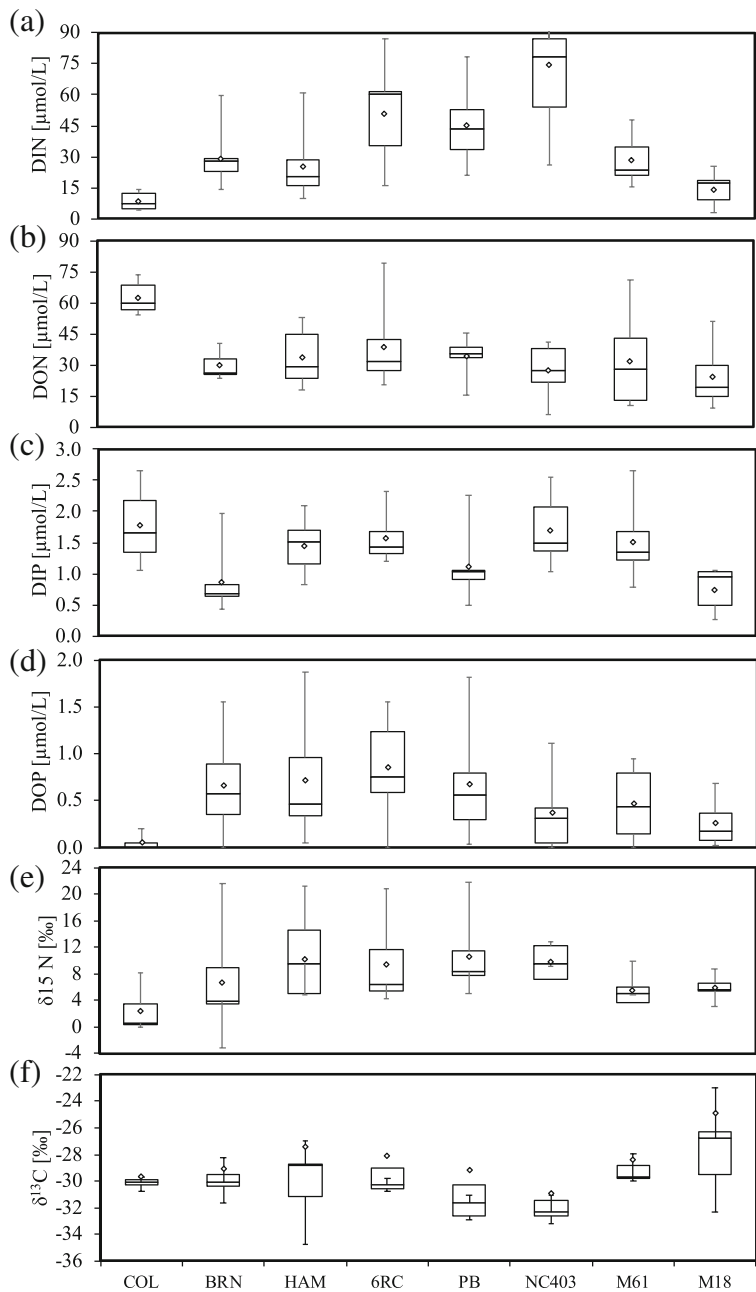
Isotopic signatures

$\delta^{15}\text{N}$ POM isotopic signatures ranged from -3.60 to $+21.7\%$ (Table 3; Figs. 2e, 6, and 7). Isotopic signatures at COL never reached above $\delta^{15}\text{N} + 10\%$, except for one outlier of $\delta^{15}\text{N} + 10.5\%$ in October 2017 (Table 3; Figs. 2e, 6, and 7). M18 $\delta^{15}\text{N}$ signatures also remained below $+10\%$ (Figs. 2e and 6). Station M61 had one $\delta^{15}\text{N}$ isotopic signature that was $+9.9\%$ (Figs. 2e and 6). Stations that showed several instances of isotopic signatures above $\delta^{15}\text{N} + 10\%$ include PB, NC403, BRN, HAM, and 6RC (Figs. 2e, 6, and 7). CAFO site 6RC and point-source site PB had particularly elevated mean $\delta^{15}\text{N}$ signatures at $+10.6$ and $+10.5\%$ (Table 3). Average $\delta^{15}\text{N}$ signatures at both point-source sites PB and NC403 were significantly ($p < 0.05$) higher than those of COL, M61, and M18; $\delta^{15}\text{N}$ signatures at CAFO sites 6RC and HAM were significantly higher than COL. The highest individual $\delta^{15}\text{N}$ signature was station PB in April at $+21.7\%$ (Figs. 2e, 6, and 7). BRN closely followed at a maximum $\delta^{15}\text{N}$ of $+21.6\%$ in April (Table 3). HAM and 6RC also had $\delta^{15}\text{N}$ signatures above $+20\%$ at $+20.1\%$ in August and $+20.9\%$ in April (Figs. 2e, 6, and 7). Point-source site NC403 also showed heavy $\delta^{15}\text{N}$ signatures at $+12.8$ and $+12.3\%$ in May and June (Table 3). Overall, $\delta^{15}\text{N}$ signatures above $+10\%$ generally had the highest values in April and occurred either in the summer months of May, June, or August (Figs. 2e, 6, and 7).

POM signatures of $\delta^{13}\text{C}$ ranged from -32.7 to -20.2% (Table 3). The control site COL generally had a narrow $\delta^{13}\text{C}$ isotopic range of -30 to -29% (Table 3; Figs. 2f, 6, and 7). M18 had isotopic signatures that ranged from $\delta^{13}\text{C} - 30.1$ to -20.2% (Table 3). HAM also exhibited a broad range of $\delta^{13}\text{C}$ signatures from -31.3 to -22.7% . PB, BRN, 6RC, COL, and M61 all had isotopic signatures ranging from $\delta^{13}\text{C} - 31$ to -27% , with one outlier point at $\delta^{13}\text{C} - 31.3\%$ (Figs. 2f, 6, and 7). Overall, COL had the most specific $\delta^{13}\text{C}$ signature and the other stations varied temporally and spatially.

As mentioned, normalized data were subject to correlation analyses (n for all correlations below was either 53 or 54). The overall $\delta^{15}\text{N}$ signature was positively correlated with nitrate concentration ($r = 0.34$, $p = 0.01$) and DIN concentration ($r = 0.29$, $p = 0.04$), but was uncorrelated with ammonium concentration (Table 4). $\delta^{15}\text{N}$

Fig. 2 Box-Whisker plots (minimum, maximum, 75th, 25th percentile, average (open circle), and median) **a** dissolved inorganic nitrogen (DIN), **b** dissolved organic nitrogen (DON), **c** dissolved inorganic phosphorus (DIP), **d** dissolved organic phosphorus (DOP), **e** stable nitrogen, and **f** stable carbon isotopic signatures in POM for the study duration (February through August, 2018)



signatures were not correlated with rainfall or discharge. Among nutrient concentrations, nitrate was negatively correlated with DON ($r = -0.44, p < 0.01$). Ammonium was not significantly related to organic nutrients, but was positively correlated with DIP ($r = 0.36, p < 0.01$). Rainfall was not significantly correlated with inorganic nutrients, but was negatively related to DON ($r = -0.38, p < 0.01$). River discharge was negatively related to DIP ($r = -0.46, p < 0.01$).

Discussion

Spatial nutrient variability

Nutrient concentrations (TN, TP, DON, DOP, $\delta^{13}C$, $\delta^{15}N$) of streams throughout the Cape Fear River Basin vary by CAFO influence. COL drains largely undeveloped wetlands with little CAFO influence from the watershed and was used as a baseline comparison against

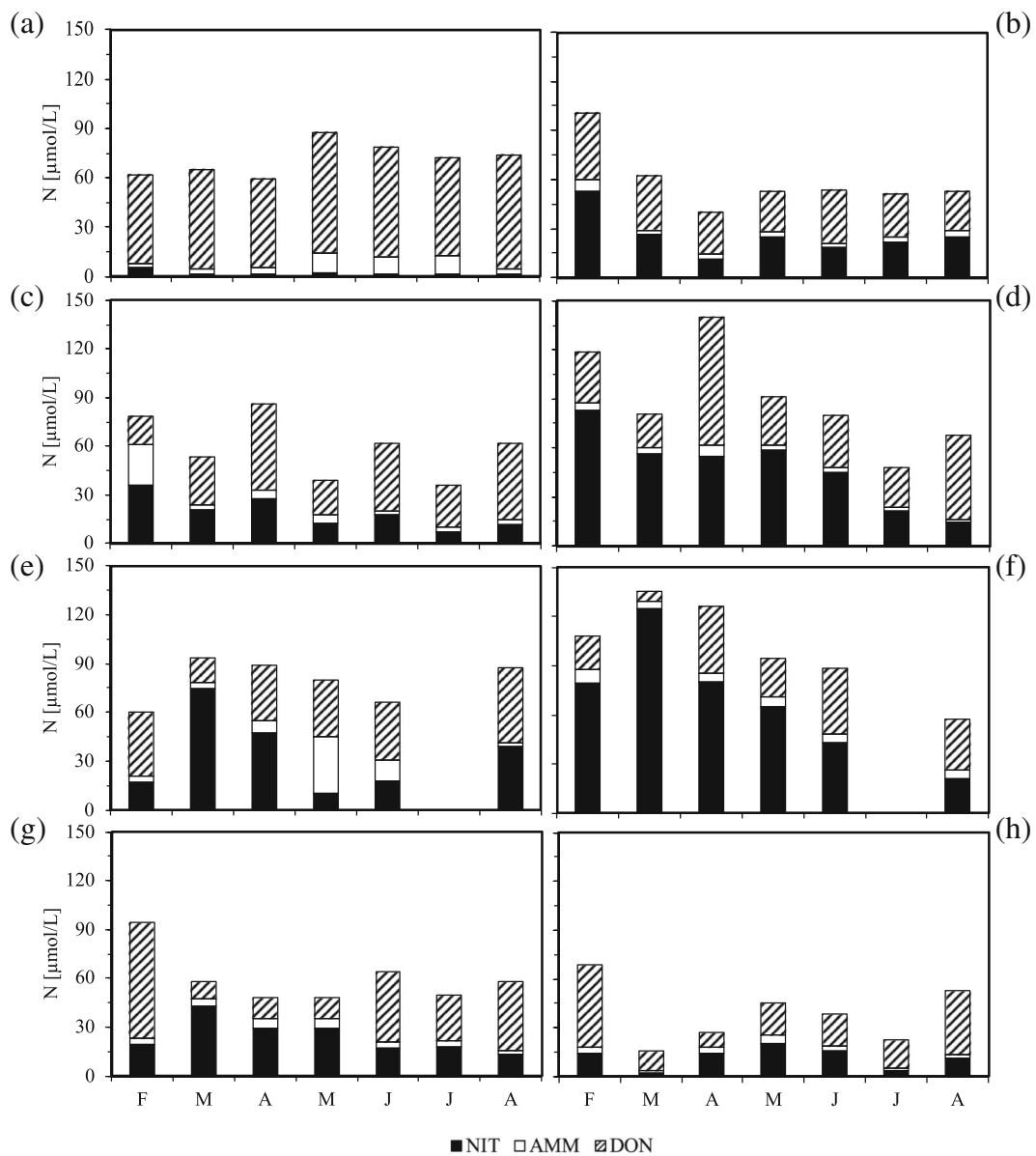


Fig. 3 Total dissolved nitrogen (TDN) concentrations are comprised of dissolved inorganic nitrogen (DIN), in the form of nitrate (NIT; $\text{NO}_3^- + \text{NO}_2^-$) and ammonium (AMM; NH_4^+), and dissolved organic nitrogen (DON). The bars in this figure depict the

concentrations of DIN and DON for comparison at each station, **a** COL, **b** BRN, **c** HAM, **d** 6RC, **e** PB, **f** NC403, **g** M61, and **h** M18, for the study duration, February through August of 2018. No samples were collected in July from PB and NC403

anthropogenically influenced sites. Though COL has one less CAFO in its direct vicinity than BRN, it is considered unimpacted because BRN's watershed drains the residential and commercial area of Elizabethtown. The consistently low nitrate concentrations ($2.24 \pm 1.33 \mu\text{mol L}^{-1}$) at COL exemplify unimpacted black water systems that have characteristically low nitrogen concentrations as nutrients are not preserved in the

floodplain and soils have low N concentrations (Smock and Gilinsky 1992; Mallin et al. 2006). The wetland-rich watershed would be conducive to enhanced denitrification. Total nitrogen values of unimpacted blackwater systems are predominately DON, which was demonstrated at COL with DON concentrations that significantly exceeded concentrations at all other sites (Figs. 2b and 3a–h). The data set as a whole demonstrated a

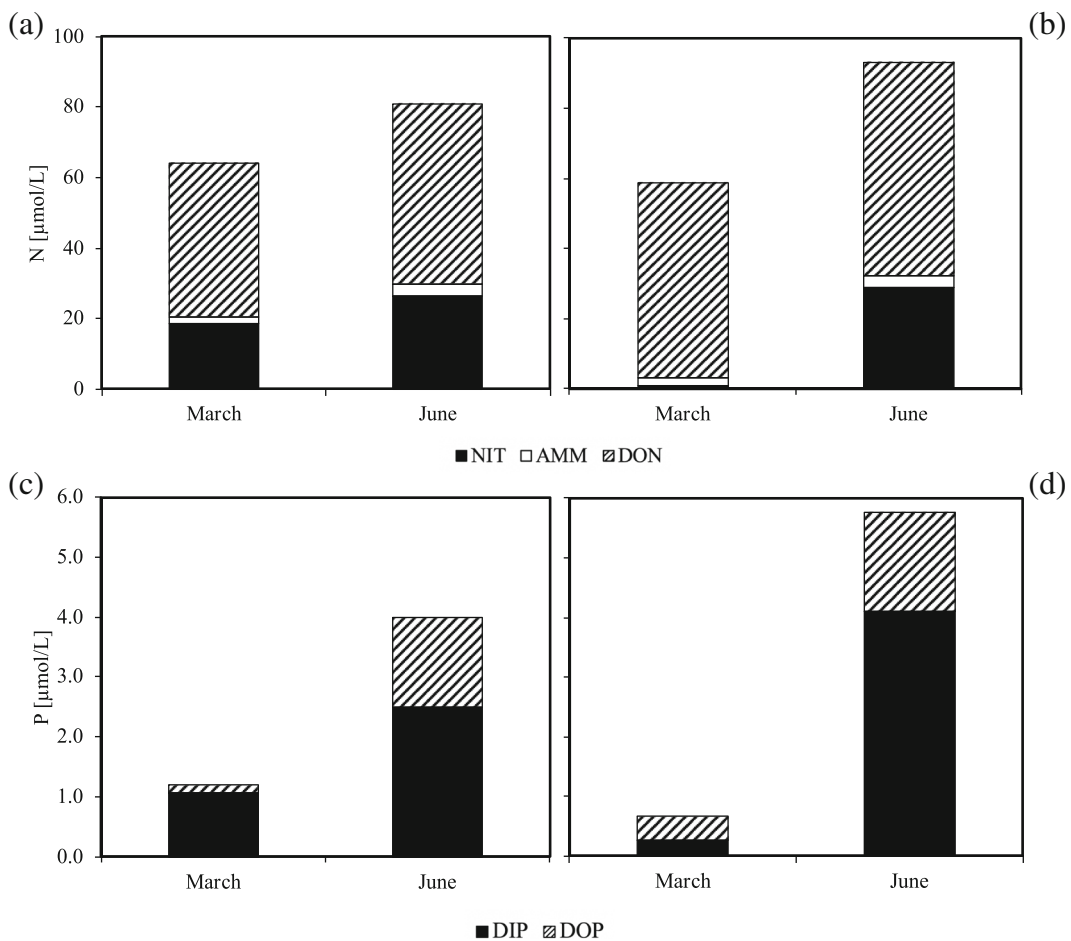


Fig. 4 Nutrient concentrations (N and P) are plotted for CAFO sites, Stocking Head Creek (a, c) and Poultry (b, d), prior to lagoon waste being sprayed (March) and after spray (June) of 2018

negative correlation between nitrate and DON; note that nitrate is anthropogenically sourced, while DON can be a product of wetland processes. The majority of total phosphorus concentrations were as DIP (orthophosphate), as DOP levels were nearly undetectable at COL with only one sample detectable at 0.20 μmol L⁻¹ (Figs. 2c, d, and 5). Isotopic signatures at COL commonly did not show any influence of animal effluent (δ¹⁵N < +10‰), which further exemplified the near-unimpacted condition of this stream. Feral swine have been photographed by the research team in the vicinity of this sampling site and the single abnormally high δ¹⁵N could be attributed to these swine. Additionally, the δ¹³C values were consistently ~ 29.5‰, which is indicative of the C3 plants that inhabit a freshwater system such as COL (Fry 2006).

The other freshwater sites displayed inorganic N concentrations and isotopic signatures suggestive of animal effluent and/or human wastewater. CAFO-rich

6RC had elevated DIN and DOP (Fig. 2a, d) an enriched mean δ¹⁵N of +10.6‰ and a maximum of +20.9‰ (Table 3). High DIN concentrations also characterized NC403 (Fig. 2a) which had a mean δ¹⁵N of +9.7‰ and maximum of +12.8‰ (Table 3). This station drained a watershed containing several CAFOs and a human NPDES point-source wastewater discharge (Table 2) The low variability among δ¹⁵N signatures at NC403 (Table 3) argues for strong watershed human influence (unfortunately the Cape Fear Basin has no TN or TP discharge limits—only the ammonium discharges are limited). The other NPDES site, PB, had elevated DIN and heavy δ¹⁵N signatures (Table 3). Thus, both sites with human wastewater influence showed both elevated DIN and heavy δ¹⁵N signatures, as did CAFO-rich 6RC.

The other site showing heavy δ¹⁵N mean, median, and maximum values was HAM, which has a considerable number of swine and poultry CAFOs in its

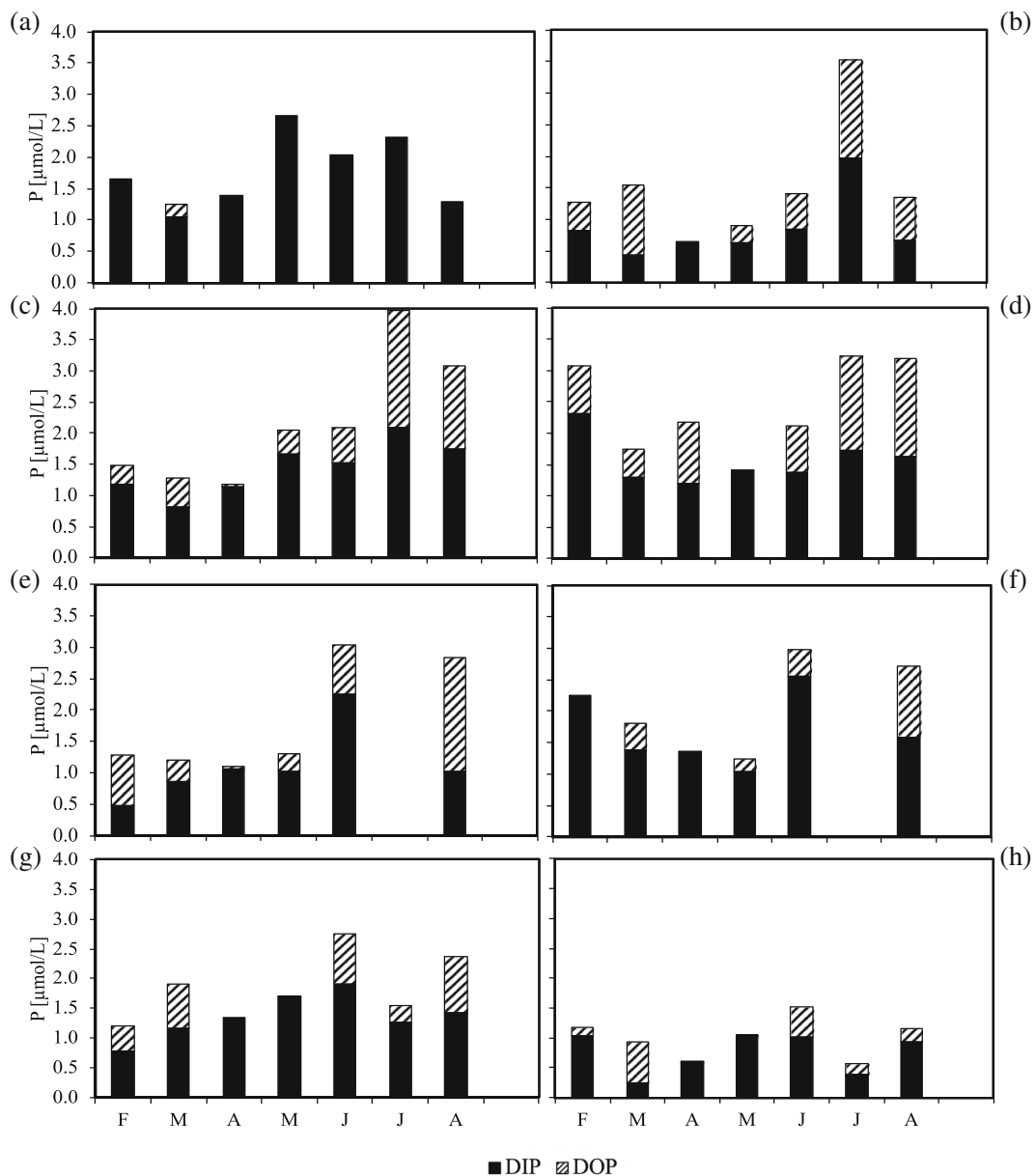


Fig. 5 Total dissolved phosphorus (TDP) concentrations, in the forms of inorganic (DIP) and organic phosphorus (DOP) are shown for each site **a** COL, **b** BRN, **c** HAM, **d** 6RC, **e** PB, **f** NC403, **g** M61, and **h** M18. The TDP concentrations are plotted

for each sampling month (February through March, 2018) in the study duration. The bars in each table represent DIP as orthophosphate (PO_4^{3-}) and DIP, to represent TDP. No samples were collected in July from PB and NC403

watershed (Table 2). In contrast, BRN had widely variable $\delta^{15}\text{N}$ signatures, with a low median (3.9) and a moderate mean $\delta^{15}\text{N}$ signature of 6.7 (Table 3). This watershed contained three CAFOs but also drains the town of Elizabethtown, presumably introducing urban and suburban stormwater runoff into Browns Creek. Note that the town's wastewater treatment plant (1.2 MGD, secondarily treated) outfall discharges directly

into the Cape Fear River. Urban stormwater runoff will contain a mixture of fertilizer N, atmospheric N, and nutrients from pet waste. The two estuarine sites showed low to moderate DIN concentrations (Fig. 2a) and lighter $\delta^{15}\text{N}$ signatures (except for April—see below).

CAFO source sites, SHC and POULT, showed nutrient concentrations and isotopic signatures that distinguished them from the other streams in the Northeast

Table 3 Isotopic signatures (‰) of the eight primary sampling sites (as mean ± standard deviation/median, range), *n* = 8 months

Site	$\delta^{13}\text{C}$ signature	$\delta^{15}\text{N}$ signature
COL	-29.7 ± 0.4 -29.7, -30.3 to -29.0	3.3 ± 4.1 1.6, -0.3 to 10.5
NC403	-30.9 ± 1.2 -30.6, -32.7 to -29.7	9.7 ± 2.8 9.4, 6.9 to 12.8
PB	-29.2 ± 1.4 -29.0, -30.9 to -27.8	10.5 ± 6.0 8.3, 4.6 to 21.7
BRN	-29.1 ± 1.2 -29.0, -31.4 to -27.4	6.7 ± 7.9 3.9, -3.6 to 21.6
HAM	-27.4 ± 2.7 -28.7, -30.6 to -22.7	10.0 ± 7.3 9.5, 0.4 to 21.1
6RC	-28.2 ± 0.9 -27.7, -29.4 to -27.2	10.6 ± 6.8 6.3, 3.3 to 20.9
M61	-28.4 ± 1.1 -27.9, -30.6 to -27.6	5.4 ± 2.3 5.1, 3.5 to 9.9
M18	-25.0 ± 3.2 -25.7, -30.1 to 20.2	5.9 ± 1.9 5.5, 2.9 to 8.8

Cape Fear River basin. Swine and poultry-influenced SHC had a $\delta^{15}\text{N}$ signature characteristic of swine effluent (+ 18.2) in March and a mixed animal waste signature of + 7.4‰ in June, and POULT showed poultry waste signatures (+ 6.6–7.0‰) in March and June (Wassenaar 1995). Sprayfields and lagoons for swine CAFOs were adjacent to SHC, while POULT was sampled at a perennial ditch draining a large poultry CAFO. The waste disposal system at poultry farms utilize dry litter applied on fields that pose different environmental impacts than hog farms as there is no liquid waste

spraying unless the poultry CAFO is an egg-laying facility; there are no NPDES requirements for dry-disposal poultry CAFOs. Dry poultry litter can impose adverse environmental effects from field runoff in rain events or light litter particles carried by wind into adjacent waterways, which increase nutrient concentrations.

A broad-based USGS study found substantially higher nitrogen concentrations (nitrate, ammonium, and TN) in swine and poultry-intensive watersheds compared to those without influence from these agricultural facilities (Harden 2015). The Cape Fear River watershed has significant spatial variability, where stations with high-density CAFO influence (as well as sites with NPDES point-source discharges) had higher nutrient concentrations and $\delta^{15}\text{N}$ isotopic signatures indicative of waste effluent than that of the relatively unimpacted blackwater system. While the Cape Fear watershed has considerable acreage under crop agriculture, the positive correlation between nitrate concentrations and heavier $\delta^{15}\text{N}$ signatures suggests that animal waste as well as human wastewater play a stronger role in nitrogen pollution than crop fertilizers. We also note that under conditions of elevated streamflow (see next section) swine waste effluent from upstream regions of the watershed can influence the water quality in downstream estuarine regions that have no direct influence of CAFOs.

Seasonal variability

CAFOs follow a seasonal schedule for waste disposal (spraying), and the transitions through the seasons are shown in each sample site’s nutrient concentration and isotopic composition, dependent on the proximity to

Fig. 6 Particulate organic matter (POM) isotopic signatures plotted for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at each sampling station from February to August, 2018

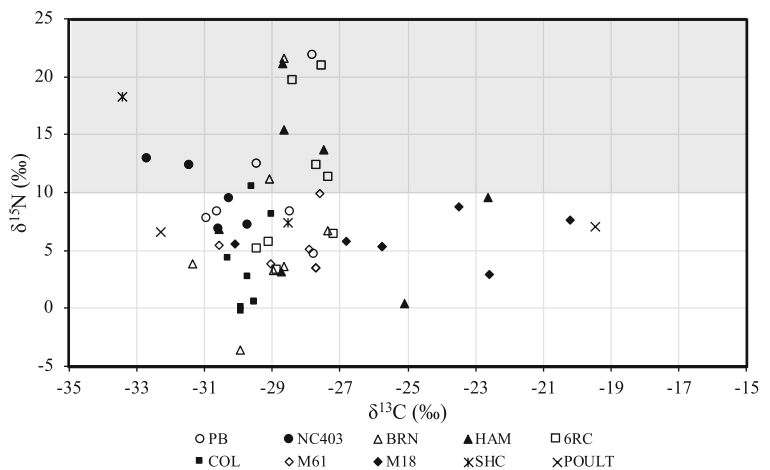
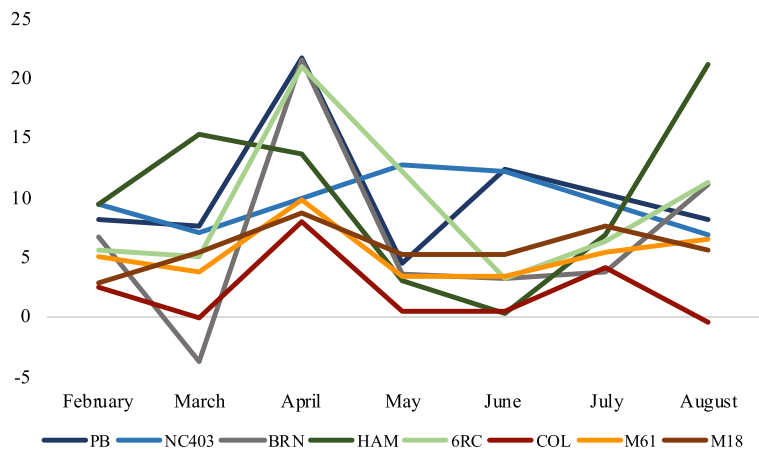


Fig. 7 Monthly variability of isotopic $\delta^{15}\text{N}$ signatures for each sampling station



CAFOs. The spraying season for swine farms to relieve waste lagoons onto surrounding Bermudagrass fields occurs during the growing season, from March to September. It was previously demonstrated that nitrate, total nitrogen, and fecal bacteria concentrations in CAFO-rich stream stations rose considerably after the onset of spray season in relation to before (Mallin and McIver 2018). Thus, it was expected for months following the start of spraying season in March to exhibit elevated nutrient concentrations and isotopic signatures characteristic of swine effluent, compared to the fall and winter months exclusive of spraying. The Bermudagrass fields, where waste is sprayed on, are intended to sequester the excess nutrients introduced by swine effluent. Bermudagrass has one of the highest capabilities to uptake nutrients, and thus was chosen as a cover crop on which to spray lagoon waste. However, as shown in this study and others (Harden 2015; Mallin et al. 2015; Mallin and McIver 2018), high concentrations of nutrients still enter adjacent waterways during the spraying

season, although in the present study 6RC and NC403 showed decreased concentrations after the initial spraying. The maximum practical nitrogen application rate for Bermudagrass in coastal areas is suggested to be 300 pounds per acre per year, divided into three application times (Conrad-Acuña et al. 2019). This application rate is solely for the maximum growth of Bermudagrass and does not assess the environmental factors of N leaching into the surrounding environment. Overapplication of waste or leaching from applying the maximum N application rate may explain the increased nutrient concentrations. The threshold of nutrient sequestration was likely met in the Bermudagrass field, and excess nutrients leach into groundwater or run off over land into surrounding waterways.

Isotopic signatures were not notably characteristic of swine effluent in March; however, in April, there was a large and widespread increase as $\delta^{15}\text{N}$ signatures amplified at some sites to +21.0‰, which is highly indicative of manure-sourced effluent. 6RC, a CAFO-dominated tributary with 179 swine CAFOs, also exhibited its heaviest $\delta^{15}\text{N}$ signature in April at +20.9‰ (Fig. 7). In fact, average $\delta^{15}\text{N}$ signatures for the entire sampling set were significantly ($p < 0.05$) higher in April compared to all other months sampled. Nitrogen signatures may not have been expressed in March due to the lag time of leachate to enter waterways. Enriched $\delta^{15}\text{N}$ signatures in April may be explained by the demand for farmers to rid an entire winter's worth of waste from the lagoons in March. In April, hydrological conditions changed that favored distribution of swine waste-derived nitrogen well downstream. Local rainfall was elevated; for instance, the total April rainfall at the Wilmington airport was 13.6 cm, which was 6.5 cm

Table 4 All significant correlations between measured parameters ($\delta^{15}\text{N}$, DIN, DON ammonium, nitrate, DIP, discharge, and rainfall)

	$\delta^{15}\text{N}$	DON	Ammonium	Discharge
Nitrate	$r = 0.34$ $p = 0.01$	$r = -0.44$ $p < 0.01$		
DIP			$r = 0.36$ $p < 0.01$	$r = -0.46$ $p < 0.01$
DIN	$r = 0.29$ $p = 0.04$			
Rainfall		$r = -0.38$ $p < 0.01$		

over the long-term April average. In addition, monthly Cape Fear River discharge at Lock and Dam #1 (Fig. 1) rose to 2170 CMS, the highest discharge of 2018 until August (2562 CMS). The presence of accumulated spray field nutrients, elevated rainfall, and high river discharge carried swine-sourced N well downstream into the estuary.

Enriched $\delta^{15}\text{N}$ signatures at BRN were shown in April (+21.6‰) and again in August (+11.2‰). Additionally, HAM showed enriched $\delta^{15}\text{N}$ signatures in March, April, and August (Figs. 2e, 6, and 7). A lack of effluent signature mid-spraying season (May through July) suggests that waste lagoons were pumped down at the beginning of the spraying season and again later in the season (note the elevated rainfall in August would lead to increased pump-out). It was interesting to note that March $\delta^{15}\text{N}$ isotopic signatures at BRN were characteristic of synthetic fertilizer at -3.6‰, the lightest signature occurring in the data set. As a predominantly rural region, inputs could have originated from surrounding traditional agricultural farms; also note that stormwater runoff from Elizabethtown would be factor as well. The nitrogen concentrations (nitrate, ammonium, and DON) at BRN, HAM, 6RC, and NC403 increased in February. Maximum nitrate concentrations are typical in winter/early spring months in watersheds containing crop agriculture from runoff, whereas summer nitrate concentrations in CAFO areas are generally significantly higher due to waste application on sprayfields (Mallin and McIver 2018).

Previous studies have traced nutrient sources with isotopic techniques and documented limited downstream nutrient transport and (Karr et al. 2001). The present study traced the potential presence of upstream CAFO-derived nitrogen downstream from CAFO-influenced areas to estuarine station M61 at least on one occasion. As previously noted, the stream and river sites had nitrogen signatures characteristic of swine effluent in April (+10 to +2 0‰), and estuarine sites M61 and M18 also exhibited unusually elevated signatures ($\delta^{15}\text{N}$ +9.9‰ and +8.8‰, respectively) in this month of elevated river discharge (Fig. 7). A clear regulatory application from these data is that waste application from lagoons in the spraying season have shown significant month-to-month variability within the basin, which indicates that a singular month of sampling to uphold state and federal water quality regulations would not be truly suggestive of potential environmental threats.

Regulations and impact of findings

Swine-derived isotopic signatures throughout the Cape Fear River basin support the findings of previous studies that showed elevated nutrient concentrations related to CAFO-impacted streams in the Cape Fear River basin. Elevated nitrogen concentrations and isotopic signatures characteristic of swine effluent have entered surface waters throughout the Cape Fear River watershed, which under the Clean Water Act G.S. 143-215.10E deems discharge of waste in surface waters illegal. CAFO facilities that intend to discharge waste into waterways are issued NPDES permits to mitigate and control waste discharge. However, only 14 of 1222 registered CAFOs claim to discharge waste into waterways and have obtained these permits. Presence of waste-derived nutrients throughout the watershed suggests that more than 14 CAFO facilities have waste that is discharged into surrounding waterways that have not obtained permitting. Nevertheless, violations of this regulation suggest that there is an enforcement issue with NPDES permits. A solution to this is mandated NPDES permits for all facilities which will aim to alleviate nutrient pollution in waterways and adverse human health impacts. In addition, a current permit monitoring only includes sampling lagoon waste to uphold regulatory standards; however, adjacent stream quality assessments should be required with permits to ensure waste is not illegally leeching into surrounding waterways. The permitting requirements for animal waste discharges vary considerably across states and suffer from lack of required stream water sampling (Rosov et al. 2020). Finally, the two point-source influenced stations in the data set displayed both elevated DIN concentrations and enriched $\delta^{15}\text{N}$ signatures in stream sites well downstream of the facilities, demonstrating that wastewater treatment in generally rural watersheds contributes to problematic nutrient loading as well as animal waste discharges.

Conclusions

Nutrient concentrations, isotopic signatures, and correlation analyses indicated that nitrogen inputs in the Northeast Cape Fear River, Black River, and the Cape Fear River are largely derived from CAFO swine effluent, with additional contributions from human discharges. CAFO-derived nutrients from watershed

tributaries, under appropriate hydrological conditions (i.e., periods of elevated rainfall during swine waste spray season), can be traced downstream as far as the Cape Fear estuary near the City of Wilmington, NC. Spatial variability was shown throughout the basin as the unimpacted blackwater and estuarine systems revealed significantly lower N concentrations and lighter isotopic waste signals than animal waste and human wastewater-impacted waterways upstream. Significant seasonal variability was identified as months with waste application to spray fields (March through August) had maximum nitrate concentrations and isotopic signatures indicative of waste effluent. Adjustments to current regulations enforcing pollution permits or monitoring of adjacent streams could aid in management and overall health of basin's waterways and the community that uses it.

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