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PRIMARY RESEARCH PAPER

Nutrients in precipitation and the phytoplankton responses to enrichment in surface waters of the Albemarle Peninsula, NC, USA after the establishment of a large-scale chicken egg farm

Karen L. Rossignol · Hans W. Paerl · John M. Fear · Jeremy S. Braddy

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Abstract Nitrogen (N) and phosphorus (P) released in waste from animal feeding operations can stimulate phytoplankton biomass production in local receiving waters. Changes in weekly wet atmospheric N and P were measured from 2005 to 2008 at monitoring stations located 0.8, 7.9, and 10.3 km downwind from a new chicken egg production facility on the Albemarle Peninsula, North Carolina. After this farm began operating, there was a significant doubling in mean wet NH_4^+ concentrations (465–1,022 μg l^{-1}) at 0.8 km with no change at the other sites. To measure the phytoplankton responses to nutrient enrichment, we conducted seasonal N and P enrichment bioassays from 2006 to 2008 on nearby Phelps Lake and Alligator River. These low-nutrient waters responded to nutrient additions, with the highest increases in phytoplankton primary productivity (^{14}C uptake) and biomass (chlorophyll *a*) occurring in the combined N and P treatments suggesting co-limitation of N and P. Although we did not find an increased nutrient signal in precipitation at sites >0.8 km from the farm, there is the potential for atmospheric deposition of N to these and other waters located N/NE of the farm given prevailing winds and distance that NH_4^+ aerosols can travel. Furthermore, surface runoff from the farm may impact receiving waters downstream (e.g., Pungo and Pamlico Rivers). In order to prevent excessive phytoplankton productivity and biomass both N and P inputs should be carefully assessed and potentially controlled in these nutrient-sensitive waters.

Keywords Nitrogen · Phosphorus · Water quality · Precipitation · Bioassay · Albemarle

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K. L. Rossignol (✉) · H. W. Paerl · J. S. Braddy
Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC 28557, USA
e-mail: krossign@email.unc.edu

J. M. Fear
North Carolina National Estuarine Research Reserve, North Carolina Division of Coastal Management, Beaufort, NC 28516, USA

Introduction

Nitrogen (N) and phosphorus (P) are key nutrients that control phytoplankton primary production and biomass in aquatic ecosystems (Hobbie, 2001; Wetzel, 2001). As urban, industrial, and agricultural development have increased in the last century, so have nutrient over-enrichment in these ecosystems (Vollenweider, 1982; Nixon, 1995). Increasing nutrient inputs have stimulated growth of phytoplankton in receiving waters, which have led to declines in water quality due to increased algal bloom formation, hypoxia/anoxia, and fish kills (Paerl, 1985, 1995;

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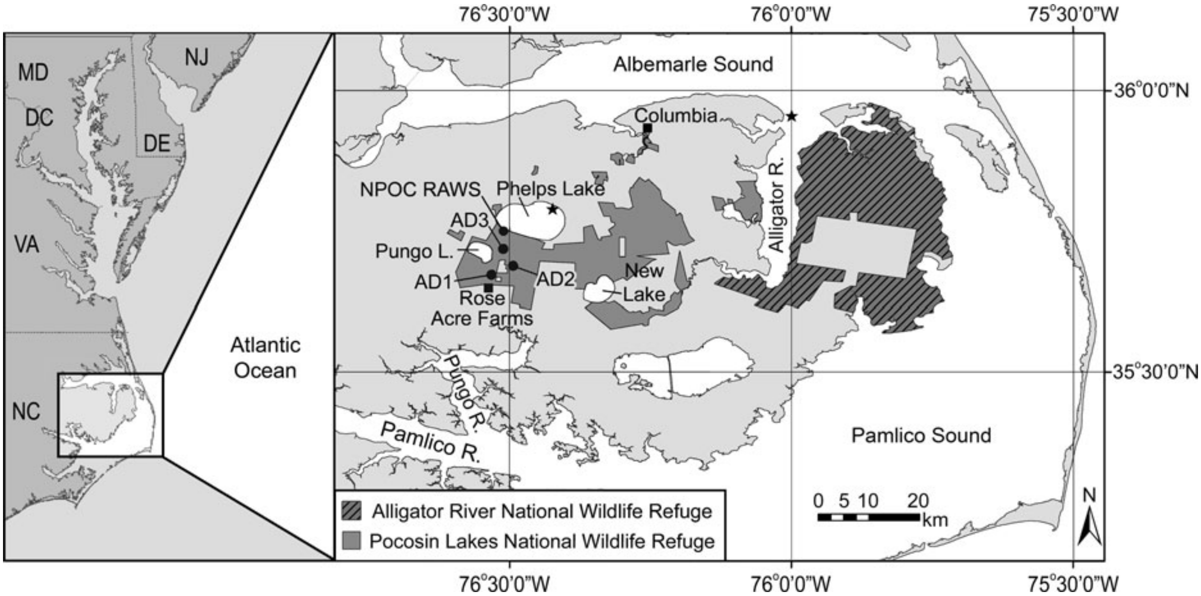

Fig. 1 Location of Albemarle Peninsula in Eastern North Carolina which includes: Phelps Lake, Pungo Lake, New Lake, Pungo River, Pamlico River, Alligator River near Alligator River National Wildlife Refuge, Rose Acre Farms, the three wet precipitation monitoring stations (AD1, AD2, and AD3) on Pocosin Lakes National Wildlife Refuge and weather station NPOC RAWS. *Stars* represent the approximate sampling locations for bioassays

Nixon, 1995; Boesch et al., 2001; Paerl et al., 2002; Paerl & Piehler, 2008).

Confined animal feeding operations can be large sources of nutrients to local receiving waters (Tarkalson & Mikkelson, 2003). Waste from feeding operations (e.g., waste treatment lagoons, confinement houses, and spray irrigation) has intensified the release of both N and P via leaching and runoff into local ground and surface waters. Nutrients, especially N, can also be released into the atmosphere. Most is released from these operations in the form of ammonia (NH_3), which when dissolved in rainwater is largely converted to ammonium (NH_4^+) (Stephen & Aneja, 2008). It is estimated that up to 55% of the total NH_3 emissions in the United States comes from such animal feeding operations, 15% of which is contributed by poultry (chickens and turkeys) (USEPA, 2005). Domestic animal operations in North Carolina (NC) are the largest statewide contributor of NH_3, particularly in the Coastal Plain portion of the state, which supported over 8 million hogs in this region during 1996 (Walker et al., 2000).

The Albemarle-Pamlico Sound (APS) (Fig. 1) is the USA's second largest estuarine system and is highly sensitive to N enrichment (Copeland & Gray, 1991; Rudek et al., 1991; Boyer et al., 1994; Paerl et al., 2006). Atmospheric inputs of N were estimated to provide >30% of the calculated 23 million kg annual N-load to APS (Fogel & Paerl, 1993; Paerl et al., 1997; Whitall et al., 2003; USEPA, 2000). The shallow, depressional wetlands, or Carolina Bay Lakes, located in this region (e.g., Phelps Lake, Pungo Lake, and New Lake) are typically rain and groundwater-fed (Fig. 1) (Schalles & Shure, 1989; Lide et al., 1995; Pyzoha et al., 2008; Stroh et al., 2008). Although N input budgets are not currently available for these lakes, rainfall is an important source of water and atmospheric nutrient input is likely to have a substantial impact on these receiving waters.

The Albemarle Peninsula is the largest peninsula in Eastern NC and is located between the Albemarle and Pamlico Sounds (Fig. 1). There are multiple agricultural operations on this peninsula that could be a potential source of nutrients to receiving waters. Among these, a new egg production facility, Rose Acre Farms, was the focus of this study. This farm is located within 5 km of the Pungo River, a tributary to the Pamlico River and Sound, within 12 km of Phelps Lake and within 50 km of Alligator River, a major tributary of the Albemarle Sound (Fig. 1). This proximity suggests that the APS and several Carolina

Bay Lakes within the region may be receiving increased nutrients from Rose Acre Farms. The farm began stocking chickens in July 2006 and by April 2009 the number of chickens increased to 3 of the 4 million permitted capacity (S. Ward, US Fish and Wildlife Service, Raleigh, NC, personal communication). Since animal excreta are rich in both N and P, the waste released by this facility is a potentially important source of nutrients (especially NH_3). For example, the wash-water that the farm uses to rinse manure from the egg shells is aerated and then applied to croplands. When fully operational, this facility is expected to generate 0.54 kg of NH_3 each year per animal, or about 2.2 million kg of NH_3 per year (USEPA, 2002).

The key objectives of this study were to determine the temporal and spatial patterns of N and P in precipitation as egg production at Rose Acre Farms increased and to estimate the phytoplankton responses to nutrient enrichment in nearby receiving waters. Nutrient concentrations in precipitation were measured weekly during a 3-year period (2005–2008) at three monitoring stations near Rose Acre Farms. To evaluate the potential impact of this new nutrient source on aquatic ecosystems of the Albemarle Peninsula, we initiated a 2-year (2006–2008) study using in situ bioassays that quantified phytoplankton responses (primary productivity and photopigment-based biomass) to nutrient enrichment at two study sites. We hypothesized that there would be a significant increase in nutrient concentrations in precipitation at monitoring sites downwind from the farm. Based on results from other NC estuarine and coastal systems (Rudek et al., 1991; Paerl et al., 1995, 1997), we also hypothesized that additions of both N and P, reflecting atmospheric nutrient enrichment, would stimulate phytoplankton primary production and biomass.

Materials and methods

Site descriptions

Water for the enrichment bioassays was collected from two sites near Rose Acre Farms: Phelps Lake and Alligator River. Phelps Lake is NC's second largest natural lake (NC Division of Parks & Recreation, 2009). It is located on the Albemarle Peninsula and is adjacent to the Pocosin Lakes National Wildlife Refuge (PLNWR) (Fig. 1). This 6,000 + ha rain-fed Carolina Bay lake has an average depth of 1.4 m and is 8 km in width. It has an average elevation of 3 m and is surrounded by surface canals from which water drains away from the lake. The lake is oligotrophic and the waters are clear (NC Division of Parks & Recreation, 2009). The Alligator River is located on the Albemarle Peninsula in Dare and Hyde Counties (Fig. 1). It is a tributary of the Albemarle Sound and is connected to the Pungo River via the Intracoastal Waterway. This river forms the western boundary of the Alligator River National Wildlife Refuge (ARNWR) which covers about over 60,000 ha (US Fish & Wildlife Service, 2004) and receives both direct rainfall and runoff from streams. The Alligator River system is an important nursery for a wide variety of fish and wildlife; both PLNWR and ARNWR contain over 80,000 ha of wildlife habitat and wetlands (Albemarle Environmental Association, 2007).

Precipitation chemistry

National Atmospheric Deposition Program (NADP) approved rainfall collectors were placed on PLNWR so that nutrient concentrations in precipitation could be estimated. NADP is a long term, nation-wide (USA) atmospheric deposition monitoring program started in 1977 (http://nadp.isws.illinois.edu/). The three wet precipitation monitoring stations, AD1, AD2, and AD3 (Fig. 1), were installed between June and August, 2005. Station AD1 was closest to Rose Acre Farms (0.8 km to the NNE), station AD2 was 7.9 km to the NE of the farm and AD3 was 10.3 km NE of the farm and closest to Phelps Lake ($\sim$1.7 km away) (Fig. 1). Given these distances, AD1 was used as a proxy for deposition at the farm and AD3 as a proxy for deposition at Phelps Lake. These monitoring stations were chosen because they were located downwind of the farm (given prevailing wind patterns). Both AD1 and AD2 were bucket collectors and AD3 was a chimney-style collector in Pettigrew State Park just South of Phelps Lake (35.7373°N, 76.5149°W). Stations were sampled weekly from June 2005 to October 2008 with only a few interruptions in data collection. Operation of sample collectors followed protocols used by NADP (1999, 2000). For the purposes of this study, the June 2005 to June 2006 sampling period will be referred to as the period before the farm began operating and July 2006 to October 2008 as the period after the farm began operating.

Precipitation samples were analyzed for dissolved inorganic N (NH_4^+ and NO_x (NO_2^- + NO_3^-)), and dissolved phosphate (PO_4^{3-}). Raw weekly nutrient concentrations were log transformed to improve normality for statistical analyses. Either a one-way analysis of variance (ANOVA, $\alpha = 0.05$) or a Kruskal–Wallis non-parametric one-way rank ANOVA ($\alpha = 0.05$; when equal variance test failed) was performed followed by the Holm–Sidak post-hoc comparison to determine significant differences in nutrient concentrations between sampling periods and among stations.

Dominant wind direction on the Albemarle Peninsula was determined using daily average wind measurements from 2005 to 2008 (with a lapse in data from Nov 2006 to Aug 2007) obtained from the State Climate Office of NC (http://www.nc-climate.ncsu.edu/) at weather station NPOC RAWS (Pocosin Lakes Remote Automated Weather Station) located within 1 km South of Phelps Lake (35.75°N, 76.51°W) (Fig. 1).

Nutrient bioassays

Five nutrient addition bioassays were conducted seasonally over a 2-year period (2006–2008) on water obtained from Phelps Lake (sample location: 35.78813°N, 76.41301°W) and Alligator River (sample location: 35.94244°N, 75.9852°W) (Fig. 1). The first bioassay in June 2006 was the only experiment conducted before the arrival of chickens at Rose Acre Farms. Water samples were collected at a depth of approximately 0.5 m and were transported to the nearby PLNWR office located in Columbia, NC to incubate in the Scuppernong River under ambient light and temperature conditions similar to sampling sites (except for October 2008 when water was incubated in an outdoor pond at the UNC Institute of Marine Sciences in Morehead City, NC) (Rudek et al., 1991; Mallin & Paerl, 1992). Water was collected from each site using 20 l polyethylene carboys and then distributed into 4 l polyethylene cubitainers (Hedwin Co.) which are approximately 80% transparent to photosynthetically active radiation (PAR; 400–700 nm). PAR was measured daily throughout the incubation using a LICOR LI-190SA quantum sensor.

Treatments for the in situ bioassays included: I. Control (no nutrient addition). II. Dissolved inorganic N (DIN) additions: 140 μg N-NH_4 l^{-1}, 140 μg N-NO_3 l^{-1} and 70 μg N-NH_4 l^{-1} + 70 μg N-NO_3 l^{-1}. III. Dissolved inorganic P (DIP) addition: 155 μg P-PO_4 l^{-1}. IV. Combined DIN and DIP additions: 140 μg N-NH_4 l^{-1} + 155 μg P-PO_4 l^{-1}, 140 μg N-NO_3 l^{-1} + 155 μg P-PO_4 l^{-1} and 70 μg N-NO_3 l^{-1} + 70 μg N-NH_4 l^{-1} + 155 μg P-PO_4 l^{-1} (Rudek et al., 1991). These N and P concentrations reflected the range of concentrations in previously measured riverine discharge to the Pamlico Sound system (Paerl et al., 2001; Christian et al., 2004). Four replicate cubitainers were utilized for each treatment. Nutrients were added at the start (T0) and at 48 h (T1) (except for June 2006) to avoid nutrient limitation during the incubation period. The cubitainers were incubated and subsampled for up to 96 h (T2), which provided sufficient time to measure phytoplankton growth, while minimizing container artifacts (Paerl & Bowles, 1987; Paerl et al., 1995). To avoid dissolved inorganic carbon (DIC) limitation, 0.8–2.0 mg C l^{-1} (as $NaHCO_3$) was added to all the cubitainers at the start of the bioassays.

Each cubitainer was sub-sampled at T1 and T2 for determination of nutrient concentrations and primary productivity. DIN (NH_4^+ and NO_x), total dissolved N (total N) and PO_4^{3-} concentrations were determined on glass fiber (GF/F) filtered subsamples using high-sensitivity automated colorimetric flow injection analyses (Lachat QuickChem 8000, Lachat Instruments, Milwaukee, WI, USA). Primary productivity was estimated as follows using the ^{14}C method (Paerl, 2002). In the morning (~11 a.m.) following sample collection, quadruplicate light and a single dark 76 ml polyethylene bottles containing sample water were injected with 0.3 ml of $NaH^{14}CO_3$ (ICN Radiochemicals; specific activity 58 mCi $mmol^{-1}$) and incubated with neutral density screening just below the water surface for 3–4 h. At the completion of incubation, the particulates in each bottle were collected onto 25 mm GF/F filters, dried, and fumed with HCl vapors for 20–30 min to remove abiotically precipitated ^{14}C. Filters were then placed in vials containing scintillation cocktail and counted in a Beckman-Coulter LS 6500 liquid scintillation counter. Counts were converted to total CO_2 fixed using the method of Paerl (2002). Total DIC concentrations were determined using a Shimadzu TOC-5000A carbon analyzer.

Chlorophyll *a* (chl *a*; a proxy for biomass) was also analyzed on subsamples collected at T1 and T2.Chl *a* was separated and quantified by using high performance liquid chromatography (HPLC; Shimadzu model LC-20AB) equipped with a photodiode array spectrophotometric detector (Shimadzu SPD-M20AC) (Pinckney et al., 1996, 1999). Approximately 100–150 ml of sample water was vacuum-filtered using 25 mm GF/F filters. Filters were frozen and later extracted in 100% acetone, sonicated, and stored at 20°C for approximately 24 h. Extracts (200 μl) were then injected into the HPLC. The HPLC procedures are described by Van Heukelem et al. (1994) and Pinckney et al. (1996, 1998, 2001). Chl *a* was identified according to its absorption spectrum, which was determined using a commercially obtained pigment standard (DHI, Denmark).

Ambient physical and chemical conditions, including salinity, temperature, conductivity, pH, and PAR levels were measured using a Yellow Springs International (YSI; Yellow Springs, Ohio, USA) 6600 multiparameter water quality sonde. ANOVA tests ($\alpha = 0.05$), followed by post-hoc multiple means comparisons (95% simultaneous confidence intervals for specified linear combinations, by the Tukey method), were performed to determine significance of differences between nutrient treatments.

Results

Precipitation chemistry

Wind data from weather station NPOC RAWS (Fig. 1) indicated 58.9% of prevailing winds in 2005–2008 were southerly, with 30.5% coming from the SW/SSW (easterly, northerly and westerly winds accounted for 17.7, 6.9, and 16.7%, respectively). During the summer (June–August), 70.7% of all wind directions were southerly, with the dominant wind direction of SW/SSW accounting for 39.3% of all wind directions.

When weekly rain nutrient concentrations were grouped by time periods before and after the farm began operating, there was a significant increase in mean NH_4^+ concentrations by more than two-fold at station AD1 (465–1,022 μg N-NH_4 l^{-1}, one-way ANOVA, $P < 0.001$; Fig. 2a). There were no significant differences in mean NH_4^+ concentrations by time period at either AD2 or AD3 (Fig. 2b, c). In contrast, median PO_4^{3-} concentrations underwent a significant decrease after the farm began operating (one-way rank ANOVA, $P < 0.001$), but only at stations AD2 (from 5.19 to 2.52 μg P-PO_4 l^{-1}) and AD3 (from 3.59 to 1.90 μg P-PO_4 l^{-1}) (Fig. 3b, c). There were no significant differences in mean NO_x concentrations at any of the monitoring stations (data not shown).

When weekly rain nutrient concentrations were grouped by station (Fig. 4), there was a significant difference in means for NO_x and NH_4^+ (one-way ANOVA, $P < 0.001$) and in medians for PO_4^{3-} (one-way rank ANOVA, $P < 0.001$). Post-hoc multiple comparisons showed significant differences between all stations ($P < 0.05$) for weekly NH_4^+ and PO_4^{3-} concentrations, where concentrations decreased with increasing distance from the farm (Fig. 4b, c). NH_4^+ decreased from a mean concentration of 862.9 μg N-NH_4 l^{-1} at AD1 to 236.5 μg N-NH_4 l^{-1} at AD3 (Fig. 4b). PO_4^{3-} decreased from a median concentration of 5.27 μg P-PO_4 l^{-1} at AD1 to 2.35 μg P-PO_4 l^{-1} at AD3 (Fig. 4c). While NO_x also decreased, only station AD3 was significantly different from the other stations (198.3 μg N-NO_x l^{-1}, $P < 0.002$; Fig. 4a).

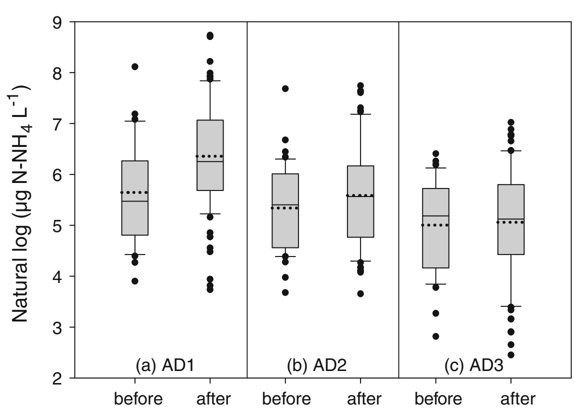

Fig. 2 Box plots showing changes in mean log-transformed NH_4^+ concentrations (μg N-NH_4 l^{-1}) before and after the farm began operating for stations **a** AD1, **b** AD2, and **c** AD3. Note the significant increase in NH_4^+ concentrations after the farm began operating for AD1. There was no significant change in NH_4^+ concentrations for AD2 or AD3. Bottom and top of boxes represent the lower and upper quartiles, respectively, the *solid line* near the middle represents the median, the *dotted line* represents the mean, whiskers (*error bars*) above and below the box indicate the 90th and 10th percentiles, respectively, and the *dots* represent the outliers

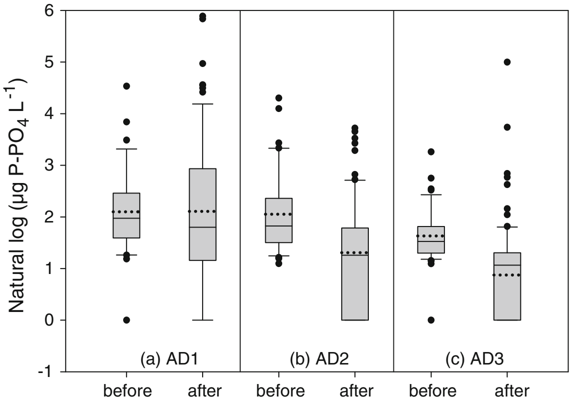

Fig. 3 Box plots showing changes in median log-transformed PO_4^{3-} concentrations (µg P-PO_4 l^{-1}) before and after the farm began operating for stations **a** AD1, **b** AD2, and **c** AD3. There was a significant decrease in PO_4^{3-} concentrations for AD2 and AD3, but no significant change in PO_4^{3-} concentrations for AD1. See Fig. 2 for description of box plots

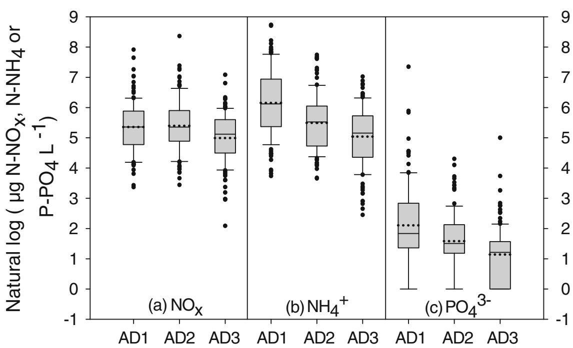

Fig. 4 Box plots showing changes in mean log-transformed **a** NO_x, **b** NH_4^+, and **c** median PO_4^{3-} concentrations (µg N or P l^{-1}) over increasing distance from farm (AD1, AD2 to AD3). NH_4^+ and PO_4^{3-} concentrations declined significantly with distance from the farm, but this decline was less evident for NO_x. See Fig. 2 for description of box plots

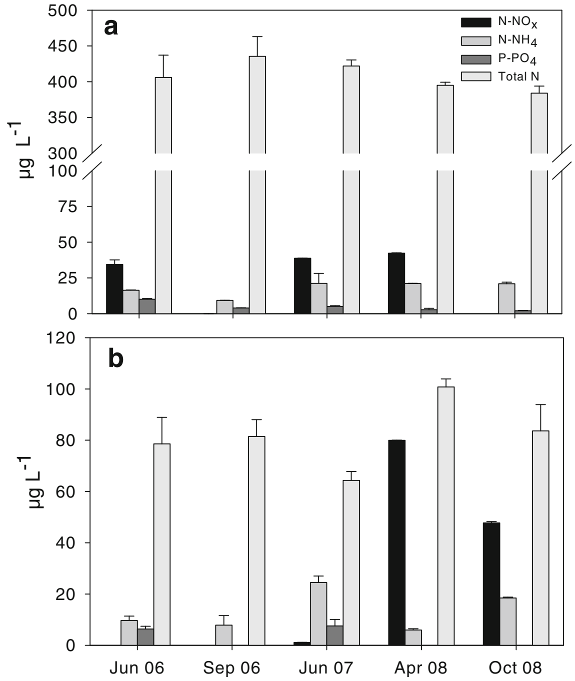

Fig. 5 Initial (T0) nutrient concentrations (µg N-NO_x, N-NH_4, P-PO_4, or total N l^{-1}) at **a** Alligator River and **b** Phelps Lake during bioassays

Nutrient bioassays

Water collected from Alligator River at T0 during the five bioassays had low-nutrient concentrations (especially PO_4^{3-}; <10.1 µg P-PO_4 l^{-1}) except for total N, which was consistently elevated in all bioassays (>384 µg total N l^{-1}) (Fig. 5a). Alligator River was oligohaline, with salinity varying from 2 to 7, and an average pH of approximately 7. In contrast, Phelps Lake was a freshwater oligotrophic system, with salinity varying from 0.05 to 0.17 and an average pH of 5.1. Water collected from Phelps Lake at T0 had relatively low concentrations of all nutrients (especially PO_4^{3-}; <7.5 µg P-PO_4 l^{-1}) (Fig. 5b). NO_x was elevated (up to 80 µg N-NO_x l^{-1}) in Phelps Lake during 2008 compared to previous years, as were NH_4^+ concentrations (>18 µg N-NH_4 l^{-1}) in June 2007 and October 2008.

Chl *a* and primary productivity in the Alligator River bioassays showed significant differences by treatments for all dates except June 2006 (one-way ANOVA, $P < 0.05$; Fig. 6). Combined DIN + DIP treatments provided the largest increase in chl *a* and primary productivity at T2 relative to controls, except for June 2006 when no significant stimulatory effect occurred. DIP additions alone never produced any stimulatory effect compared to controls in chl *a* and primary productivity. However, on two occasions, September 2006 and October 2008, DIN treatments alone significantly stimulated chl *a* and, to a lesser extent, primary productivity (Fig. 6).

In the Phelps Lake bioassays, significant differences by treatments were also observed for both chl *a* and primary productivity (one-way ANOVA, $P < 0.05$; Fig. 7). Combined DIN + DIP treatments again provided the largest increase in chl *a* and

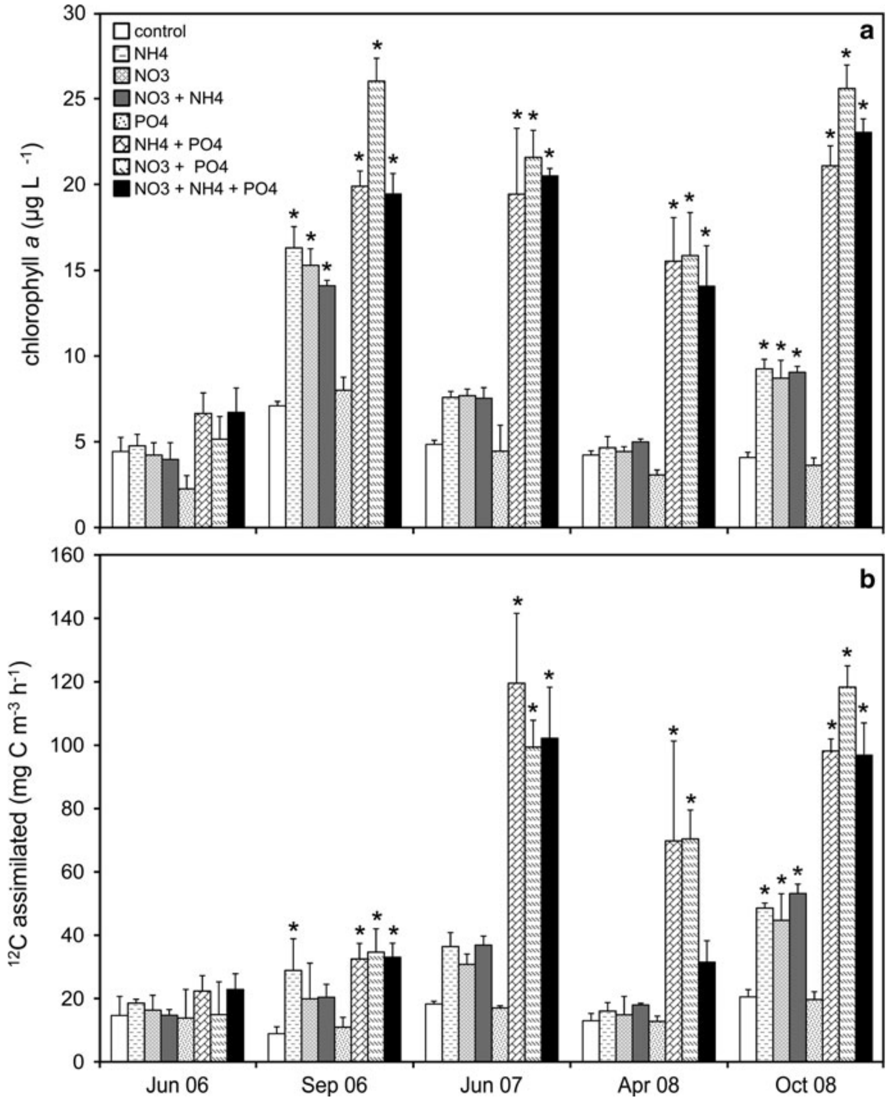

Fig. 6 **a** Chlorophyll *a* concentrations (µg l^{-1}) and **b** rates of primary productivity (^{12}C assimilated) for T2 at Alligator River. Significant differences from controls are represented by *stars* as determined by Tukey post-hoc multiple comparisons ($\alpha = 0.05$)

primary productivity at T2 relative to controls. DIP or DIN additions alone did not produce significant responses in chl *a* and primary productivity. In September 2006, there were very low (<6.7 mg C m^{-3} h^{-1}) rates of primary productivity (Fig. 7b).

Discussion

Precipitation chemistry

The increase in NH_4^+ concentrations at the station closest (0.8 km) to Rose Acre Farms suggests emissions from this facility were responsible. After the farm began operating, concentrations of NH_4^+ more than doubled over the 3-year period (Fig. 2a). Elevated NH_4^+ coincided with the increasing stock of chickens at the facility. These results are very similar to those reported by Aneja et al. (2003), who studied changes in NH_4^+ concentrations at a NADP monitoring site in Sampson County, NC. There was a dramatic increase in NH_4^+ concentrations in precipitation over an 8-year period which was associated with an increasing number of hogs in the region.

Even though increasing NH_4^+ concentrations in rainwater were evident at the station closest to Rose Acre Farms, we were not able to detect a significant change over time at stations AD2 and AD3 (Fig. 2b, c). Furthermore, there was a decrease in NH_4^+ concentrations with distance from the farm (Fig. 4b).

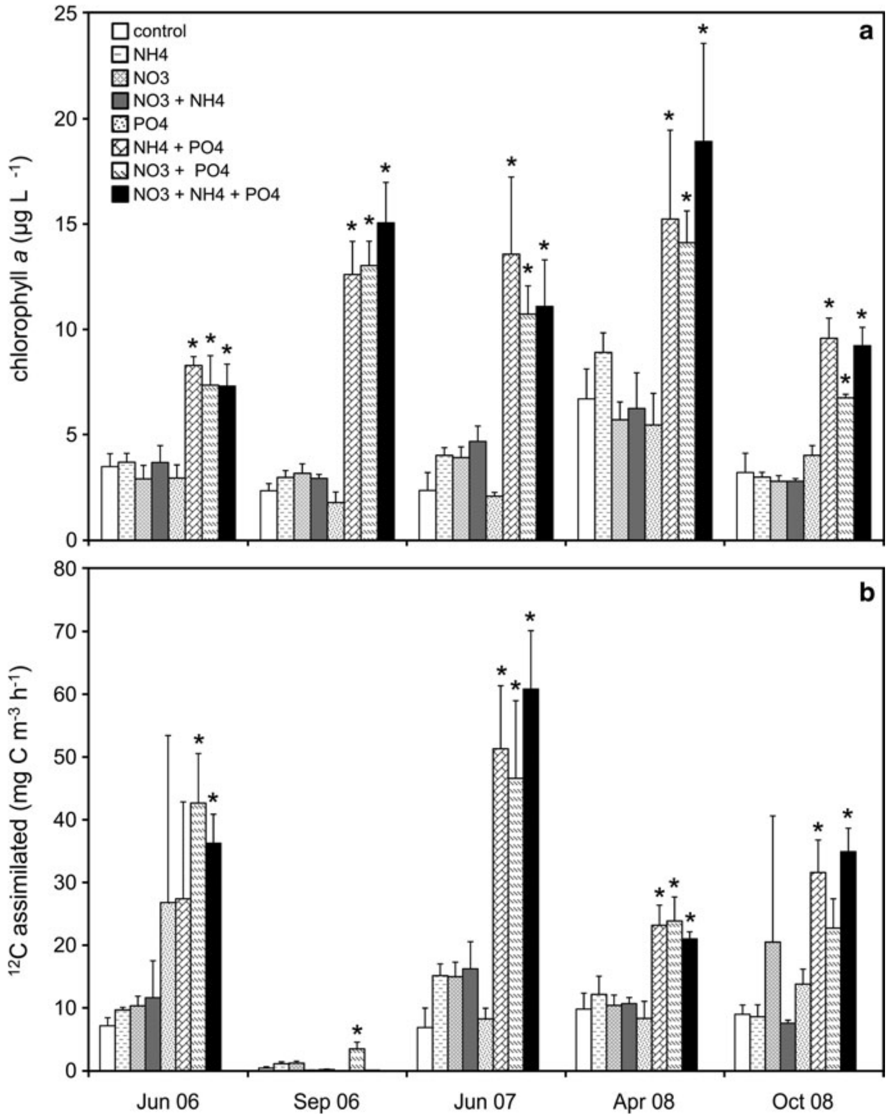

Fig. 7 **a** Chlorophyll *a* concentrations (µg l^{-1}) and **b** rates of primary productivity (^{12}C assimilated) for T2 at Phelps Lake. Significant differences from controls are represented by *stars* as determined by Tukey post-hoc multiple comparisons ($\alpha = 0.05$)

It is uncertain why we did not find an increased nutrient signal beyond station AD1 since several studies have shown that NH_4^+ aerosols can be transported 80 km or greater from their source (Irwin & Williams, 1988; Walker et al., 2000). One possible mechanism for the spatial decrease found in this study may be slow conversion rates of NH_3 to NH_4^+. When this process is slow, more N is deposited close to the emission source (Walker et al., 2000).

There were overall declines in wet atmospheric PO_4^{3-} concentrations at AD2 and AD3 after the farm began operating (Figs. 3b, c, 4c). The reason for this decline is unclear; these changes are most likely unrelated to farm emissions, especially since there was no significant change in PO_4^{3-} concentrations at the site nearest the farm (Fig. 3a). Unlike N, P does not have a stable gaseous phase in the atmosphere, and instead is associated with dust, biogenic particles and aerosols of combusted materials (Grimshaw & Dolske, 2002; Mahowald et al., 2008). It is far more likely that the P (and N) released in waste from the farm would leach into surface waters especially given the sandy soil and flat topography present in this region of NC (Tarkalson & Mikkelson, 2003).

Data reported by the NC Department of Environment and Natural Resources Division of Water Quality (DENR DWQ) also suggests that runoff is another source of nutrients from Rose Acre Farms.

Surface water samples from nine stations around Rose Acre Farms during 2005–2009 showed significant increases in mean total P concentrations after the farm began operating for both near (<0.5 km) and far (>1.5 km) stations. This pattern was also evident for all forms of N at many of the stations (J. Olinger, NC DENR DWQ, Raleigh, NC, personal communication). These data suggest that nutrient runoff from the facility could impact Pungo and Pamlico Rivers, which are located downstream (South) of Rose Acre Farms (Fig. 1). Two creeks which feed into Pungo River, Pantego and Pungo Creek, are already on the USEPA's 303(d) list of impaired waters due to elevated chl *a* levels (http://oaspub.epa.gov/tmdl/waters_list.control), likely due to excessive nutrient inputs from large agricultural drainage networks (S. Ward, US Fish & Wildlife Service, Raleigh, NC, personal communication). However, because Phelps, Pungo and New Lakes are higher in elevation compared to Rose Acre Farms, they will not receive drainage from canals that connect to the facility.

Nutrient bioassays

Bioassay primary productivity and chl *a* results clearly indicated that the waters of Phelps Lake and Alligator River respond to nutrient enrichment and that, in general, the combined effects of DIN and DIP far outweighed effects of either of these nutrients when added alone (Figs. 6, 7). Both primary productivity and biomass at these sites were co-limited by N and P. Water collected from both sites for all bioassays was relatively low in nutrients with no obvious increase in nutrient concentrations over time at Alligator River (Fig. 5a). There were periods of elevated NO_x and NH_4^+ after the farm began operating at Phelps Lake (Fig. 5b), but given the lack of elevated atmospheric nutrients at AD3 (Figs. 2c, 3c), it is difficult to link these nutrient increases at Phelps Lake to atmospheric nutrient input from the farm.

Significant differences were observed for the DIN + DIP treatments at T1 for AR (data not shown), but not at T2 in June 2006 (Fig. 6). Nutrients were not replenished during this bioassay and it is likely the phytoplankton rapidly depleted the nutrients to limiting levels by T1, leading to a cessation of new biomass production. For example, average chl *a* for the combined DIN + DIP (NO_3^- + PO_4^{3-}) treatment dropped from 18.9 μg l^{-1} at T1 to 5.2 μg l^{-1} at T2 for Alligator River. Therefore, nutrient depletion was probably responsible for the low levels of chl *a* and rates of primary productivity in the combined DIN + DIP treatments for this bioassay.

The September 2006 bioassay had very low rates of primary productivity at Phelps Lake (Fig. 7b). This bioassay was conducted 12 days following Tropical Storm (TS) Ernesto, where 223.5 mm of rainfall fell at AD3 during the first week of September. Freshwater runoff from TS Ernesto lowered DIC concentrations to near undetectable levels in water collected from Phelps Lake (0.01 mg C l^{-1}). Although $NaHCO_3$ (final concentration of 0.8 mg C l^{-1}) was added to the cubitainers, we suspect that DIC limitation may have been responsible for these low rates. Due to the buffering capacity of the higher (than Phelps Lake) salinity Alligator River, runoff from TS Ernesto had much less of an impact on DIC concentrations, and thus productivities, in this river (Fig. 6b).

If nutrient-enriched rain or surface water reached the Albemarle Peninsula's receiving waters it could result in increased phytoplankton biomass and primary production. Increased concentrations of NH_4^+ in receiving waters could have a particularly strong impact on phytoplankton biomass for it is often the preferred form of DIN (Syrett, 1981; Collos, 1989). For example, Twomey et al. (2005) found NH_4^+ to be the dominant form of N taken up by phytoplankton, contributing approximately half of the total measured dissolved N (NO_3^-, NH_4^+, urea) uptake throughout the Neuse River Estuary (a tributary to the Pamlico Sound). High inputs of nutrients may lead to increased frequency of phytoplankton blooms, which may be more likely during warmer months when NH_3 is more volatile (USEPA, 2004) and phytoplankton growth rates are often highest and most responsive to nutrient enrichment. There could be negative ecological effects if potentially harmful (i.e., toxic, food-web disrupting) bloom-forming species are favored by these nutrient inputs.

Conclusions

This study demonstrated that wet NH_4^+ concentrations increased greatly at the monitoring station closest to Rose Acre Farms after its establishment, suggesting that it is originating from this facility and is likely an important source of "new" N to the area. We were not able to find an increased nutrient signal beyond the closest station to the farm. However, given prevailing winds in the area and distance that NH_4^+ aerosols can travel, there is the potential for atmospheric deposition of N to the receiving waters located N/NE of the farm (e.g., Phelps Lake, Pungo Lake, Alligator River). Furthermore, given the extensive system of canals present on the Albemarle Peninsula, surface water runoff from the farm could be an important source of nutrients (N and P) into receiving waters located South or downstream of the farm. The effects of increasing nutrient inputs to this area could become more apparent if monitored over larger spatial and temporal scales, especially after production rates reach maximum capacity at the farm.

The bioassays conducted during this study showed that the low-nutrient waters of Phelps Lake and Alligator River responded to nutrient enrichment by increasing phytoplankton biomass and primary production. Both primary productivity and biomass at these sites were co-limited by N and P with highest increases occurring in response to combined N and P additions. In order to prevent excessive phytoplankton productivity and biomass both DIN and DIP inputs should be carefully assessed and potentially controlled in these nutrient-sensitive waters. Reducing nutrient inputs from this and other animal feeding operations will be important for the long-term protection of the Albemarle Peninsula's lakes, wetlands, and estuaries.

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